No. 2022-1761

UNITED STATES COURT OF APPEALS
FOR THE FEDERAL CIRCUIT

ABS GLOBAL, INC., GENUS PLC,

Appellants,

v.

CYTONOME/ST, LLC,

Appellee.

Appeal from the United States Patent and Trademark Office, Patent Trial and Appeal Board, in No. IPR2021-00088

BRIEF FOR APPELLANTS ABS GLOBAL, INC. AND GENUS PLC

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PATENT CLAIM

This appeal involves U.S. Patent No. 10,583,439. Claim 1 reads as follows:

> 1. A microfluidic assembly for use with a particle processing instrument, the microfluidic assembly comprising:
>
> a substrate; and
>
> a flow channel formed in the substrate, the flow channel having:
>
> an inlet configured to receive a sample stream;
>
> a fluid focusing region configured to focus the sample stream, the fluid focusing region having a lateral fluid focusing feature, a first vertical fluid focusing feature, and a second vertical fluid focusing feature, the lateral, the first vertical, and the second vertical fluid focusing features provided at different longitudinal locations along the flow channel, wherein a bottom surface of the flow channel lies in a first plane upstream of the first and second vertical fluid focusing features and the bottom surface of the flow channel shifts vertically upward to lie in a second plane downstream of the first and second vertical focusing features; and
>
> an inspection region at least partially downstream of the fluid focusing region.

(Appx95(18:43–63).)

CERTIFICATE OF INTEREST

Counsel for Appellants ABS Global, Inc. and Genus plc, Steven J. Horowitz, certifies that the following information is accurate and complete to the best of his knowledge:

1. **Represented Entities.** Provide the full names of all entities represented by undersigned counsel in this case. Fed. Cir. R. 47.4(a)(1).

   ABS Global, Inc.
   Genus plc

2. **Real Party in Interest.** Provide the full names of all real parties in interest for the entities. Do not list the real parties if they are the same as the entities. Fed. Cir. R. 47.4(a)(2).

   ABS Global, Inc.: None
   Genus plc: None

3. **Parent Corporations and Stockholders.** Provide the full names of all parent corporations for the entities and all publicly held companies that own 10% or more stock in the entities. Fed. Cir. R. 47.4(a)(3).

   ABS Global, Inc.: Genus plc
   Genus plc: None

4. **Legal Representatives.** List all law firms, partners, and associates that (a) appeared for the entities in the originating court or agency or (b) are expected to appear in this court for the entities. Do not include those who have already entered an appearance in this court. Fed. Cir. R. 47.4(a)(4).

   None.

5. **Related Cases.** Provide the case titles and numbers of any case known to be pending in this court or any other court or agency that will directly affect or be directly affected by this court's decision in the pending appeal. Do not include the originating case number(s) for this case. Fed. Cir. R. 47.4(a)(5).

Inguran, LLC v. ABS Global, Inc., No. 3:20-cv-00349-wmc (W.D. Wis.).

6. **Organizational Victims and Bankruptcy Cases.** Provide any information required under Fed. R. App. P. 26.1(b) (organizational victims in criminal cases) and 26.1(c) (bankruptcy case debtors and trustees). Fed. Cir. R. 47.4(a)(6).

Not applicable.

August 15, 2022

/s/ *Steven J. Horowitz*

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STATEMENT OF RELATED CASES

This is an appeal from an *inter partes* review of U.S. Patent No. 10,583,439 before the Patent and Trademark Office's Patent Trial and Appeal Board, with ABS Global, Inc. and Genus plc (collectively, "ABS") as petitioners, and Cytonome/ST, LLC ("Cytonome") as patent owner. No other appeal from the proceeding was previously before this or any other appellate court.

The '439 patent is also at issue in *Inguran, LLC v. ABS Global, Inc.*, No. 3:20-cv-00349 (W.D. Wis.). Counsel for ABS are unaware of any other case in this or any other court that will directly affect or be affected by this Court's decision in this appeal.

JURISDICTIONAL STATEMENT

This is an appeal from the Board's April 28, 2022 Final Written Decision. (Appx1–61.) The Board had jurisdiction under 35 U.S.C. §§ 311, 316(c). ABS timely appealed on April 29, 2022. (Appx809–811.) This Court has jurisdiction under 28 U.S.C. § 1295(a)(4)(A).

INTRODUCTION

The '439 patent relates to microfluidic devices—devices that have very small channels etched into them through which a sample of fluid containing one or more particles can flow. Fluids flowing through such channels behave in well-known ways. Most relevant here, when one fluid is flowing in a microfluidic channel and a second is injected under the proper conditions, the two fluids will not mix; instead, they will flow side by side down the channel in what is referred to as "laminar flow." Using this well-understood phenomenon, skilled artisans can inject fluids, or use channel geometry such as ramps and tapers, to confine or squeeze a sample fluid in order to adjust its shape, size, or position within a microfluidic channel. This process is known as "focusing," and it has been commercially used to help analyze, count, or sort particles (such as mammalian cells) for decades.

At a high level, the '439 patent claims a microfluidic device with several features found in textbook devices years before the patent's priority date. The claimed device has a flow channel that begins with an opening, or "inlet," that is configured to receive "a sample stream," which continues through a "fluid focusing region" that is "configured to

focus the sample stream," before ultimately reaching an "inspection region" further downstream.

The Board concluded that the main prior art reference in this case, an article referred to as "Simonnet," disclosed every limitation of the sole independent claim at issue, except one. Specifically, the Board concluded that Simonnet did not disclose "the sample stream" in the phrase "configured to focus the sample stream." In the Board's view, "the sample stream" referred to in claim 1 is singular. Based on two images of streams produced by Simonnet under certain flow conditions, the Board concluded that Simonnet taught a device that produced a split sample (*i.e.*, a sample with a gap in the middle), and a split sample, the Board reasoned, is actually *two* streams. The Board therefore concluded that Simonnet failed to teach "the sample stream" of claim 1. In short, the Board's patentability determination turned on its decision to construe "the sample stream" to require one single, contiguous sample stream throughout the device.

The Board's decision cannot stand for two reasons. First, the Board erred in construing "the sample stream" to require one single, contiguous stream. That error stemmed from the Board's

misunderstanding of the definite article "the" in "the sample stream." In that phrase, the definite article is not used to limit the *number* of streams, but instead refers back to an antecedent "sample stream" in a preceding limitation (like the common alternative "said," as in "said sample stream"). The word "the" thus provides an answer to the question, "*Which* sample stream is focused in the fluid focusing region?" The answer is the same sample—"a sample stream"—that was introduced upstream at an inlet to the flow channel. And because the antecedent "a sample stream" uses the indefinite article "a," which the patent expressly defines as plural, the claim must be read to cover a device configured to focus *one or more* sample streams. The Board was therefore wrong to require one single, contiguous stream.

Second, the Board's decision should be reversed because, regardless of how "the sample stream" is construed, Simonnet disclosed a device "configured to focus the sample stream." Simonnet is widely cited as an exemplary focusing device in the field of microfluidics; it is literally used in textbooks to teach students about such devices. If "the sample stream" is properly construed as a reference to "a sample stream," Simonnet disclosed a device configured to focus that sample.

And importantly, the claims are directed to a *device*, not a *method*, which means that what matters is whether the device has the requisite configuration, not how it is used. Thus, even if "the sample stream" were construed to mean one single, contiguous stream, Simonnet would still satisfy the limitation, as it expressly indicates that the device is configured such that no split or gap will occur under a variety of flow conditions. Moreover, even when the device does produce a split sample, that sample is *still* a single stream—just with a new feature, the gap. In all cases, Simonnet's device is configured to focus "the sample stream."

For these reasons, and as explained further below, the Board's decision should be reversed, or at a minimum, vacated and remanded.

STATEMENT OF THE ISSUES

1. Whether the Board erred in construing "the sample stream" in claim 1 to require one single, contiguous sample stream.

2. Whether, under any proposed construction of "the sample stream," the challenged claims are unpatentable.

STATEMENT OF THE CASE

A. Background on Microfluidics

The invention claimed in the '439 patent is a microfluidic device. (Appx87(1:14–19).) Microfluidics refers to the flow of fluids at very small scales through channels less than one millimeter in diameter. (Appx1983.) At such scales, fluids behave in well-understood ways. In particular, under the proper conditions, when one fluid flows through a microfluidic channel, and a second fluid is injected into the channel, the two fluids do not mix. (Appx1983.) Instead, the two fluids remain separate and flow through the channel side by side. (Appx1983.) This phenomenon is known as "laminar flow," and is illustrated in the figure below:

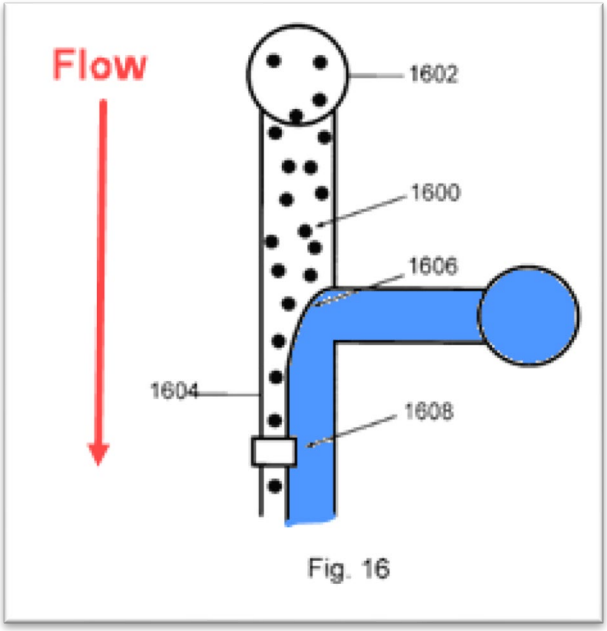


Fig. 16

(Appx4159–4160 (annotating Appx2213(fig. 16)).)

In this figure, one fluid containing small black particles (1600) flows downward through a microfluidic channel. (Appx2225(9:40–52).) At the junction, a second, blue fluid (1606) is injected into the channel from the right side. (Appx2225(9:48–51).) As the figure shows, instead of mixing with the first fluid, the second fluid remains separate and squeezes the first fluid against the left side of the channel, confining the first fluid into a narrower area. The two fluids then flow downward through the channel side by side.

For decades, skilled artisans[1] in the field of microfluidics have used this well-understood phenomenon to manipulate fluids, known as "sample fluids," that contain particles (such as cells) that need to be examined, counted, or sorted. (*See, e.g.*, Appx2138; Appx2223(5:1–5).) By squeezing or confining a sample fluid using separate fluids, known as "focusing fluid" or "sheath fluid," skilled artisans are able to "focus"

[1] Throughout this brief, ABS uses the term "skilled artisan" to mean a person of ordinary skill in the art as defined by the Board: a person with "a bachelor's or master's degree in the field of bioengineering, mechanical engineering, chemical engineering, or analytical chemistry, or a related field and at least three years of experience in designing or developing microfluidic systems." (Appx11–12.)

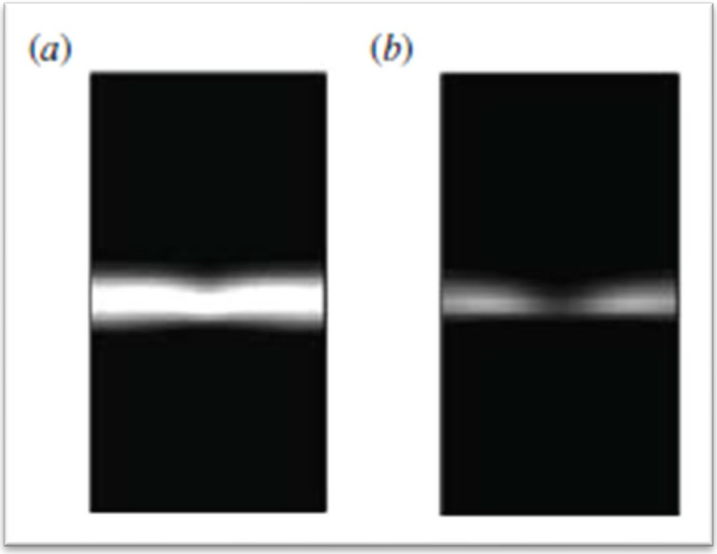
the sample fluid into a desired placement and shape within a microfluidic channel. (*See, e.g.*, Appx2148 ("The side flow squeezes, or 'hydrodynamically focuses,' the inlet flow into a thin stream.").)

Sample fluids have long been "focused" in different directions within a flow channel, such as horizontally (or "laterally") and vertically. (Appx1985; *see* Appx1985–1991.) Since the field of microfluidics took off in the 1990s, skilled artisans have developed devices (sometimes referred to as "chips"[2]) that can achieve lateral and vertical focusing for "a diverse range of applications." (Appx2778; Appx1988–1989.) Which focusing device a skilled artisan would select, and how she would use that device, would "depend on the context of a particular application." (Appx4502(346:5–10).)

Skilled artisans have long understood that the speed at which fluids within a microfluidic channel move relative to one another (known as their "relative flow conditions" or "relative flow rates") can affect how a sample fluid is focused. (*See* Appx4171–4172; Appx4174–

[2] Microfluidic devices often resemble credit-card sized pieces of glass, silicon, or plastic, and may be referred to as "chips." (*See, e.g.*, Appx2148.)

4178; Appx2782 ("In either two- or three-dimensional hydrodynamic focusing, the focused stream is always changed to the required size by adjusting the flow rate ratio of the sample to the sheath flows.").) For instance, as the flow rate of the focusing fluid relative to the sample fluid increases, the focusing fluid exerts greater pressure on the sample fluid, compressing it into a smaller cross-section. (*See* Appx2782.) This effect is shown below in Figures (a) and (b), which depict a decrease in the thickness of a sample fluid (shown in white) as the flow rate of the focusing fluid (in black) increases relative to that of the sample fluid.



(Appx2782(figs. 7(a), 7(b)).)

Figure (a) depicts a cross section of the sample fluid when the focusing fluid is moving four times as fast as the sample fluid.

(Appx2782(fig. 7(a)).) Figure (b) depicts the same cross section when the focusing fluid is moving twelve times as fast as the sample fluid. (Appx2782(fig. 7(b)).) Due to the increased relative flow rate of the focusing fluid squeezing the sample, the sample fluid depicted in white is notably thinner in Figure (b) than in Figure (a). (Appx2782 (noting that "the focused stream becomes smaller").)

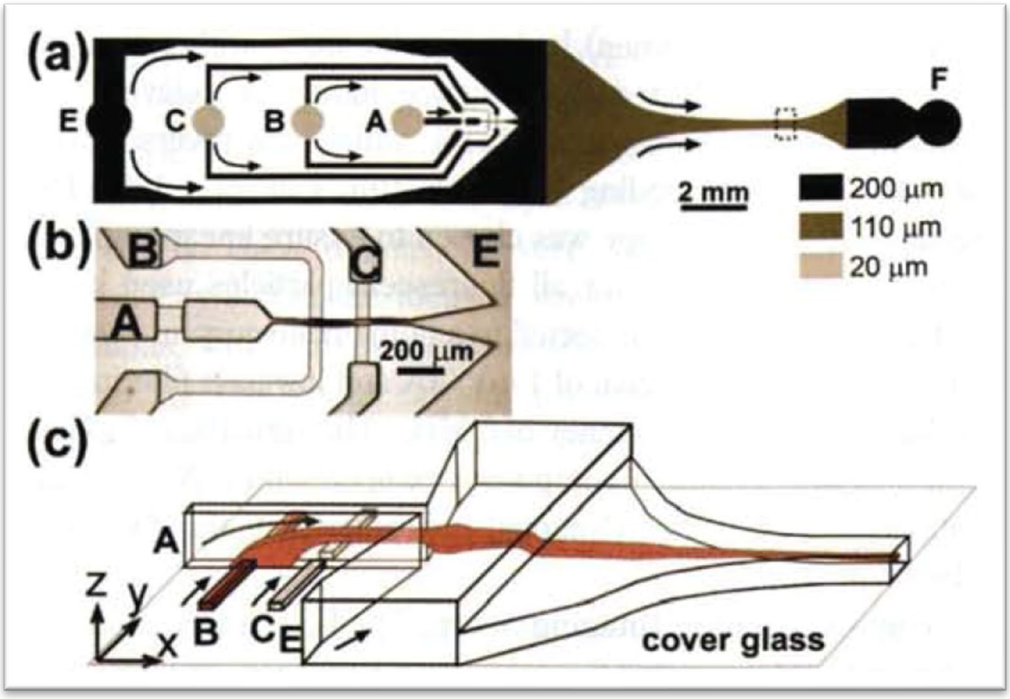
When the focusing fluid is flowing that much faster than the sample, the sample fluid depicted in Figure (b) not only shrinks in size, but actually splits, producing a "gap in the middle." (Appx2782.) As the relative flow rate of the focusing fluid increases, "this gap will become larger." (Appx2782.) Conversely, as the focusing fluid's relative flow rate decreases, the gap disappears, as in Figure (a).

B. Simonnet

The main prior art reference at issue in this case is a 2006 article by Claire Simonnet and Alex Groisman, "High-Throughput and High-Resolution Flow Cytometry in Molded Microfluidic Devices" ("Simonnet"). (Appx2110–2127.) Simonnet was published in the journal *Analytical Chemistry*, which, as Cytonome's own expert explained, is one of the top peer-reviewed journals in its field.

(Appx4076(9:8–11); Appx4077(11:6–24).) Simonnet has been widely cited by others (Appx2959; *e.g.*, Appx2819 & n.18), and has been described as teaching one of the "most advanced structures" for focusing in the field of microfluidics (Appx2139 & n.22). (*See also* Appx1995 ("Simonnet is literally a 'textbook design' in the field, in the sense that it is among the designs shown in at least one introductory textbook from 2013.").)

Simonnet disclosed several microfluidic devices that can be used "to fully control the position and size of the sample stream." (Appx2819; *see* Appx2118; Appx2036–2039.) The particular device at issue in this case is called the "High-Throughput Device," depicted below:

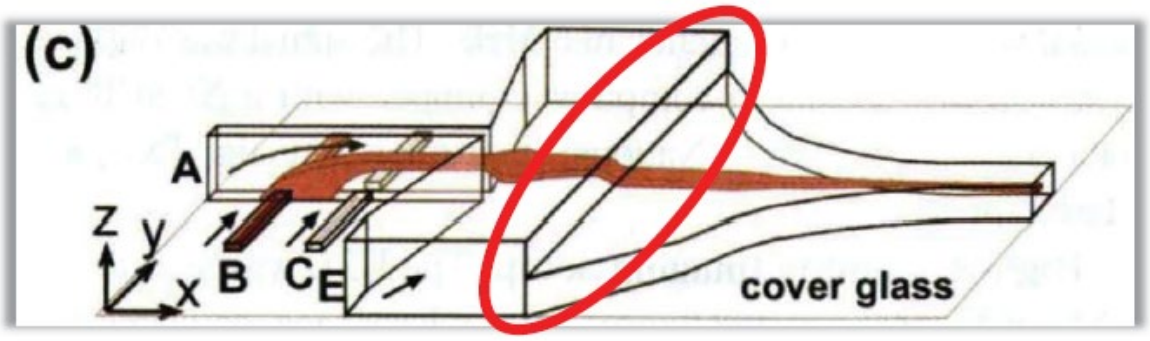


(Appx2119(fig. 1).)

As Simonnet explains, the High-Throughput Device has four entryways, or "inlets," for fluids to enter the flow channel, referred to as Ports A, B, C, and E. (Appx2118.) First, as shown in Figure (c) above, Port B can introduce a sample fluid (depicted in red) into the flow channel, which moves through the device from the left side of the figure to the right. (Appx2119; *see* Appx2042–2045.)[3] Next, Ports A and C can introduce focusing fluid above and below the sample fluid, respectively, to focus the sample in a vertical direction. (Appx2119 (explaining that fluid from Port A provides "focusing from the top" and fluid from Port C provides "focusing from the bottom"); Appx2051–2054.) In addition, Port E can introduce focusing fluid from the left and right sides of the sample fluid simultaneously, to focus the sample in a lateral direction. (Appx2119 (explaining that "flow focusing in the in-plane direction is provided by the liquid injected into port E"); Appx2050–2051.)

[3] Port B comprises two branching channels that introduce sample fluid into the primary channel from either side. (Appx2119; Appx208–209 (explaining how the sample could be understood to be introduced from a single inlet at Port B or from two inlets on either side of the primary channel).)

Then, as the figure below shows, the device contains a vertical "step" that shifts the top surface of the flow channel downward to a lower plane:



(Appx1999 (annotating Appx2119(fig. 1(c))); *see also* Appx2070–2072.[4])

Finally, at the end of the High-Throughput Device's flow channel, the sample fluid enters an inspection area where individual cells can be evaluated for relevant characteristics. (Appx2120–2121 (describing the "interrogation region, where flow cytometry assays were carried out"); Appx2074–2078.)

As is typical of microfluidic devices, variations in the relative flow rates of fluids in the High-Throughput Device can affect how a sample fluid is focused. Among other things, Simonnet notes that, at certain

[4] Such vertical shifts in a top or bottom surface of the flow channel were often used even before Simonnet. (*See* Appx1998–2001; *see also* Appx2167(fig. 5(a)); Appx2792(fig. 3).)

flow conditions, the focusing fluid from channel A will cause the sample stream to split down the middle. To characterize some of the flow conditions that might generate a focused sample stream with such a gap, Simonnet reported on experiments in which fluorescent dye and/or small plastic beads[5] were introduced into various ports of the High-Throughput Device at exemplary flow conditions.

As depicted in Figure 3(b) below, a "gap" in the sample stream "occurs when the flow emerging from channels B [*i.e.*, the sample fluid] is small compared with the flow in channel A [*i.e.*, the focusing fluid introduced from above]." (Appx2121; *see* Appx4171–4172.) When these relative flow rates are present, "the stream from inlet B is split into two parts, with a gap in the middle." (Appx2121.)

[5] These beads were used solely to measure the velocity of the focused stream. (Appx2121.) They were just 1.9 μm in diameter, much smaller than mammalian cells that the High-Throughput Device can be used to analyze, which are typically about 7 to 15 μm in diameter. (Appx2126.) In contrast, the entire stream depicted in Figures 3(a) and 3(b) is less than 10 μm in diameter. (Appx2121(figs. 3(a), 3(b)) (providing 10-μm scale); *see also* Appx4173 (illustrating 10-μm "cell" superimposed over the stream).)

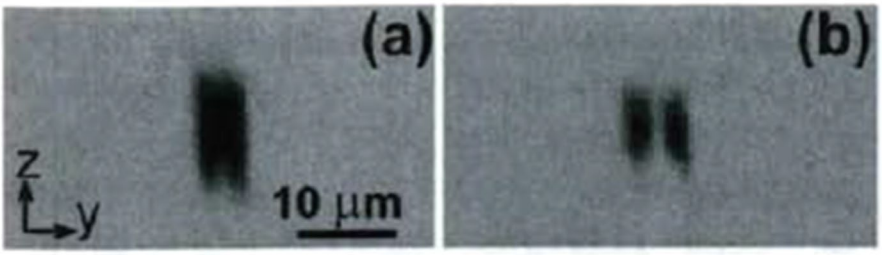


Figure 3. Confocal micrographs showing flow structure near the central axis of the cytometry channel in the HTD at conditions typical for cytometry assays. Fluorescent dye (FITCD) was injected into ports A, C, and E. Dark areas correspond to the stream from port B. Differential pressures at ports A, B, C, and E in kPa with respect to port F were: (a) 9.5, 8.9, 9.2, and 9.1; and (b) 9.5, 8.6, 9.2, and 10.2.

(Appx2121(fig. 3).)[6]

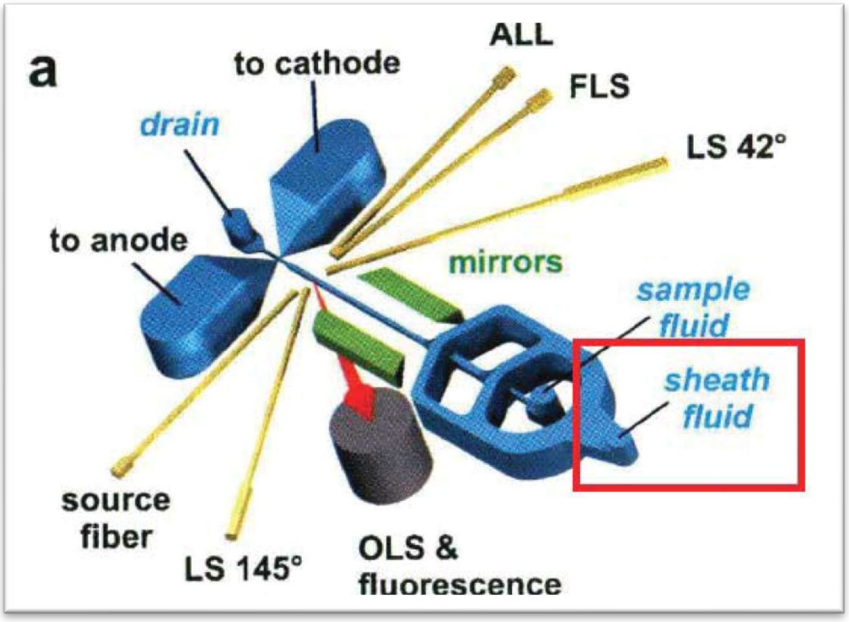
Figure 3(a), shown above, demonstrates how adjustments in flow conditions can diminish (and, at some point, eliminate) the gap. Figure 3(a) depicts a different set of flow conditions based on different experimental parameters, which included increasing the pressure applied (relative to Figure 3(b)) to introduce the sample at Port B, while maintaining the pressures applied to introduce focusing fluid at Ports A and C. (Appx2121 (reporting pressures); *see also* Appx4175–4178

[6] Skilled artisans have long recognized that certain flow conditions can produce a sample stream with a gap. (*See* Appx2782 ("This irregular shape is due to the fact that the flow rate injected from inlet A (sheath flow) is 12 times higher than that injected from inlet B (sample flow) . . . ."); *see also* Appx4164–4166.)

(explaining link between pressures and relative sheath fluid flow rates).) As a result, the flow rate of the vertical focusing fluid relative to the sample fluid decreased in Figure 3(a), compared to Figure 3(b). (*See* Appx4176–4177.) According to Simonnet, these conditions produced only a "residual gap," appearing as a small node or indent at the bottom of Figure 3(a). (Appx2121.)

C. Kummrow

Since Simonnet was published in 2006, skilled artisans have built on its design. For example, in 2009, Andreas Kummrow and several colleagues published an article called "Microfluidic Structures for Flow Cytometric Analysis of Hydrodynamically Focussed Blood Cells Fabricated by Ultraprecision Micromachining" ("Kummrow"). (Appx2128–2147.) Like Simonnet, Kummrow taught a microfluidic chip design that focuses a sample fluid vertically and horizontally. (Appx2146.) In the Kummrow device, however, focusing fluid is injected into a single inlet, which can distribute focusing fluid to different points along the flow channel through interconnected channels. (Appx2139.) Figure (a) below shows the inlet for "sheath fluid" on the bottom right-hand side of the device (labeled "sheath fluid"):



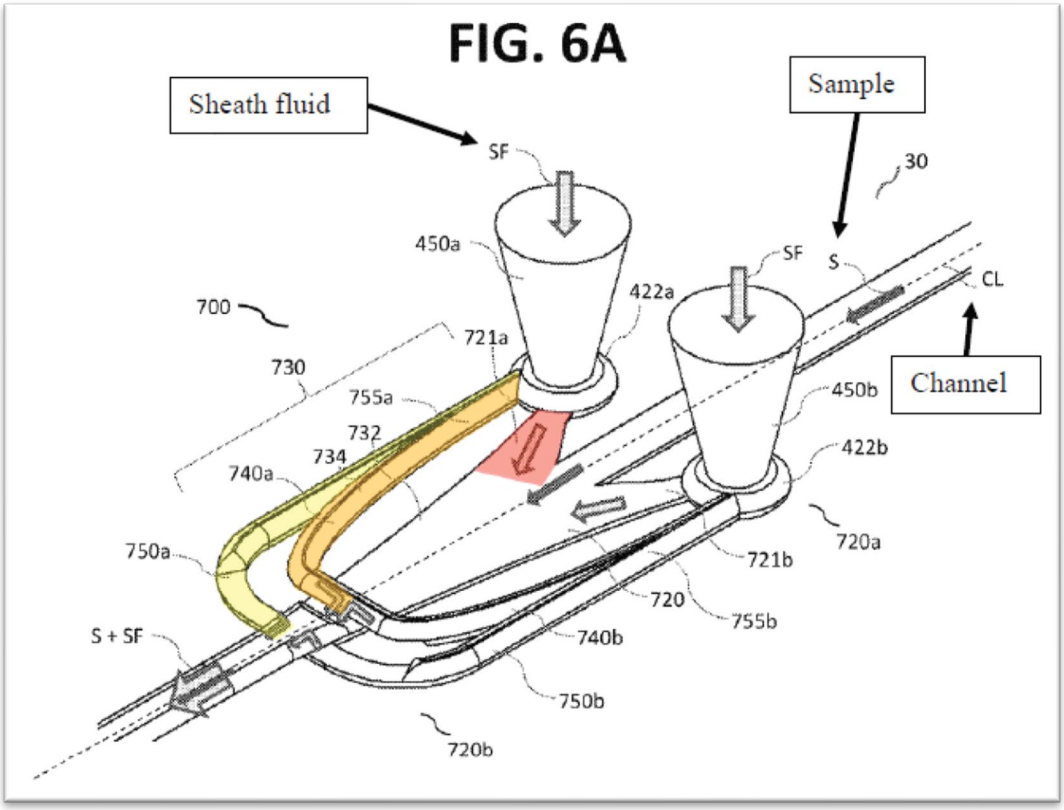
(Appx2091 (annotating Appx2139(fig. 1(a))).)

D. The '439 Patent

Years after Simonnet and Kummrow were published, Cytonome applied for the '439 patent, claiming priority to a provisional application filed on March 14, 2013. (Appx62.) The '439 patent is titled "Hydrodynamic Focusing Apparatus and Methods." (Appx62.) It discloses "[a] microfluidic chip having a micro channel for processing a sample," with several different device designs. (Appx62(abstract).) An exemplary microfluidic chip is shown in Figures 6A–6D. (Appx82–85.) In response to a restriction requirement issued during prosecution, Cytonome elected to pursue claims to the device depicted in these figures. (Appx1454–1455; Appx2012.)

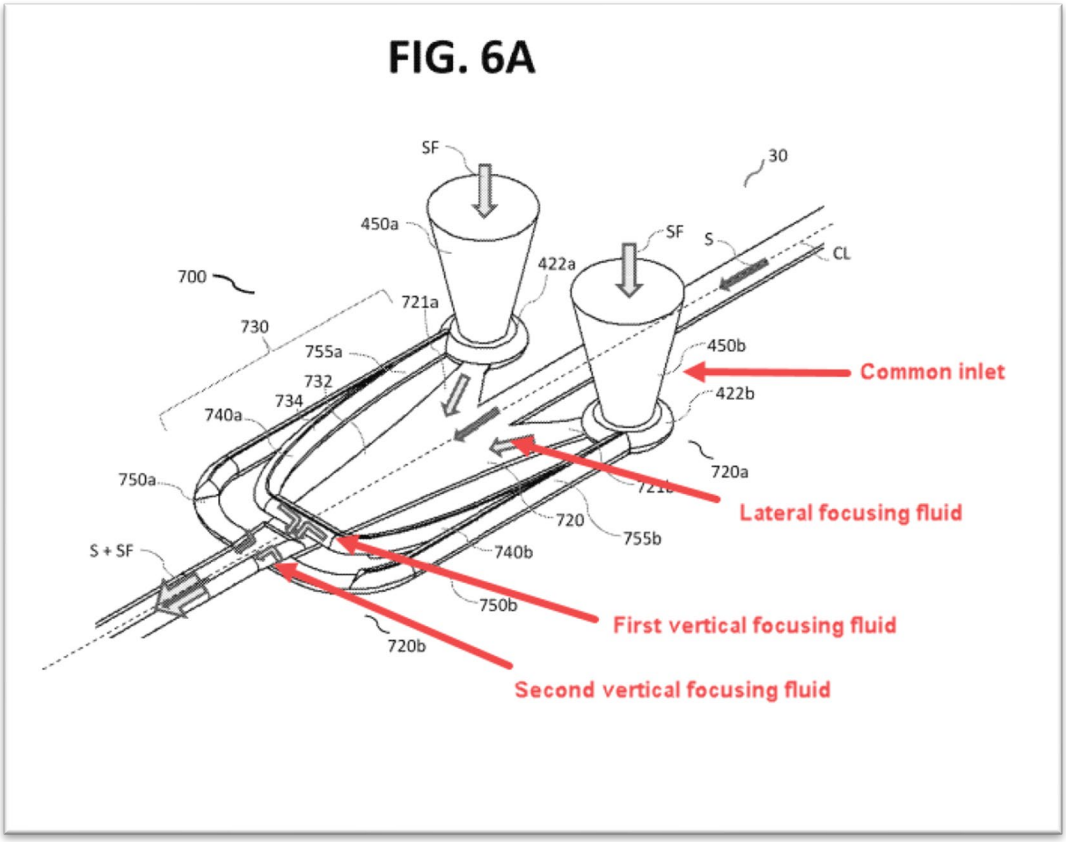
1. Device Background

Figure 6A below provides a view from above of the device described by the '439 patent. In the figure, a sample fluid flows through a channel in the device from the top right to the bottom left.



(Appx2010 (annotating Appx82(fig. 6A)); *see* Appx2009–2011.)

The flow channel then has a fluid focusing region, which contains several features capable of introducing focusing fluid to focus the sample stream. As shown below, these features can introduce focusing fluid from both lateral sides, as well as from the top and bottom.

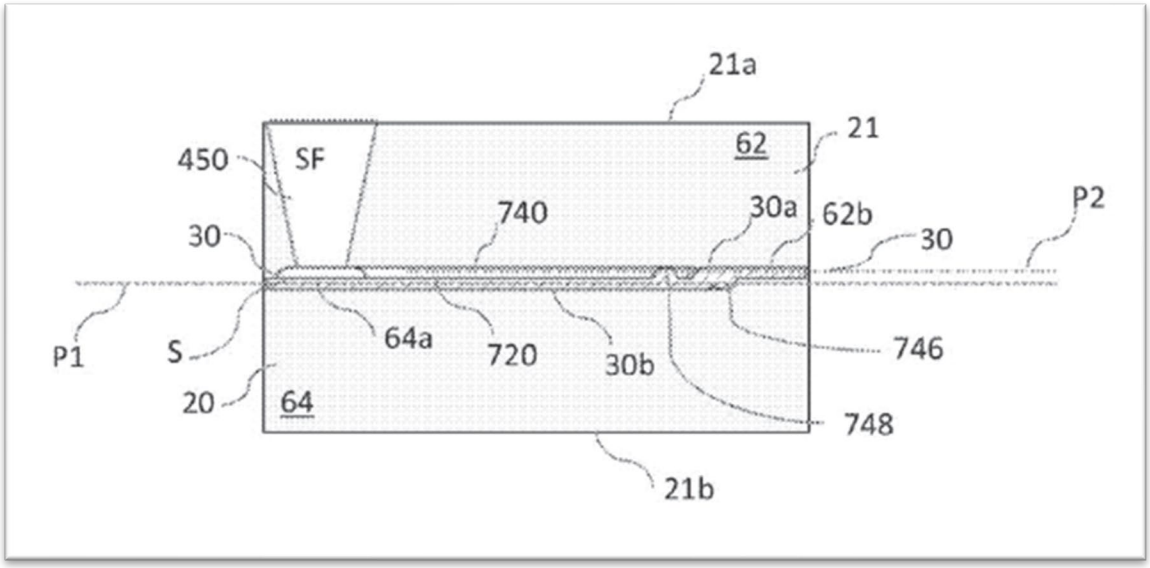


(Appx2089 (annotating Appx82(fig. 6A)).)

Finally, after the two vertical focusing features, the floor of the flow channel shifts upward, elevating into a higher plane.[7] The following figure depicts this feature, showing a cross-section of the

[7] As the '439 patent explains, directional terms like "vertical," "lateral," "top," "bottom," "above," "below," "up," and "down" are purely relative and "should be understood as descriptive terms providing general relationship between depicted features in the figures." (Appx89(6:4–10).) It was undisputed before the Board that the orientation of the device is not a claim limitation. (*See* Appx803; *see also* Appx47.)

device from the side. On the right side of the figure, the floor of the flow channel "steps up" to a higher level.



(Appx84(fig. 6C).)

Finally, the flow channel of the device ends in an inspection region. (Appx87(1:64–66).)

2. Claim Language

This appeal concerns one independent claim and four dependent claims of the '439 patent. Independent claim 1 is directed to:

> 1. A microfluidic assembly for use with a particle processing instrument, the microfluidic assembly comprising:
>
> a substrate; and
>
> a flow channel formed in the substrate, the flow channel having:

> an inlet configured to receive a sample stream;
>
> a fluid focusing region configured to focus the sample stream, the fluid focusing region having a lateral fluid focusing feature, a first vertical fluid focusing feature, and a second vertical fluid focusing feature, the lateral, the first vertical, and the second vertical fluid focusing features provided at different longitudinal locations along the flow channel, wherein a bottom surface of the flow channel lies in a first plane upstream of the first and second vertical fluid focusing features and the bottom surface of the flow channel shifts vertically upward to lie in a second plane downstream of the first and second vertical focusing features; and
>
> an inspection region at least partially downstream of the fluid focusing region.

(Appx95(18:43–63).)

The dispute in this case concerns the limitations of the "flow channel" of claim 1.

The flow channel has a number of features. First, the channel has "an inlet configured to receive a sample stream." (Appx95(18:48).) The meaning of the phrase "a sample stream"—and in particular, whether it is a singular or plural term—is important in this case. The '439 patent sheds light on that question by defining "the term 'a' or 'an' entity" to mean "one or more of that entity." (Appx95(18:27–28).) The patent explains that "the terms 'a' or 'an', 'one or more' and 'at least one' can be used interchangeably." (Appx95(18:29–30).)

After the inlet configured to receive "a sample stream," the flow channel has "a fluid focusing region configured to focus the sample stream," *i.e.*, the same sample stream that was received through the inlet. (Appx95(18:49–50).) The fluid focusing region includes "a lateral fluid focusing feature" and two "vertical fluid focusing feature[s]." (Appx95(18:50–52).) These three fluid focusing features are located in different places along the flow channel (*i.e.*, "different longitudinal locations"). (Appx95(18:54–55).) Finally, after the vertical focusing features, the floor of the flow channel elevates to a higher plane, "shift[ing] vertically upward." (Appx95(18:55–61).)

Following the fluid focusing region, the flow channel ends with an "inspection region." (Appx95(18:62–63).)

Dependent claims 2, 6, 8, and 9 of the '439 patent add requirements to the microfluidic assembly of claim 1. (Appx95(18:64–67); Appx96(19:26–29, 19:36–39, 19:40–43).) Particularly relevant to this appeal, claim 2 requires that the lateral fluid focusing feature in claim 1 be "configured to introduce focusing fluid into the flow channel symmetrically with respect to a centerline of the sample stream." (Appx95(18:64–67).)

E. Proceedings Below

ABS petitioned the Board for *inter partes* review of claims 1, 2, 6, 8, and 9 of the '439 patent. (Appx2.) ABS argued that Simonnet alone anticipates and renders obvious claims 1, 2, 6, and 8, and that Simonnet in combination with other references renders obvious claims 8 and 9. (Appx9–10.)

After the Board instituted review (Appx400–448), Cytonome's primary opposition to ABS's challenge was based on a narrow construction of the term "focusing." Cytonome argued that "focusing" should be construed so that it "necessarily requires a single stream of sample." (Appx472.) Cytonome derived this requirement from the term "sheath flow," which the '439 patent discusses in the specification, but not the challenged claims. (Appx472; *see* Appx87(1:23–25) (defining "[s]heath flow" as "a particular type of laminar flow in which one layer of sample fluid, or a particle, is surrounded by another layer of focusing fluid on more than one side").) Citing the gap depicted in Figure 3(b) of Simonnet, as well as the "residual gap" depicted in Figure 3(a), Cytonome argued that "by producing *two* sample streams, Simonnet

fails to achieve the requisite 'sheath flow' with a single layer of sample fluid and thus fails to 'focus' as claimed." (Appx487–488.)

ABS responded that "focusing" means "pinching, confining, squeezing, or constricting," and does not include a "sheath flow" or "single sample stream" limitation. (Appx518–523.) Using this construction, ABS explained that a sample stream can be focused, even if the stream is also split, citing various works by skilled artisans discussing focused sample streams with gaps or splits. (Appx521–523.) Finally, ABS pointed out that, even if focusing required a single stream, the Simonnet device can produce such a stream under various flow conditions, as the article itself explains. (Appx530–532.)

In its Final Written Decision, the Board held that none of the challenged claims are unpatentable, but not based on Cytonome's arguments around the term "focusing." In fact, the Board agreed with ABS that the term "focusing" means "pinching, confining, squeezing, or constricting," rejecting Cytonome's additional limitations. (Appx28; *see also* Appx16–20.) The Board explained that "[a]lthough sheath flow may, in certain cases, be a natural result of having multiple fluid focusing features . . . the claim itself does not require that focusing

include sheath flow." (Appx16.) Thus, the Board declined to "import additional requirements" into the term "focusing." (Appx28.)

Nevertheless, the Board imported a new requirement into an entirely different phrase: "the sample stream" in claim 1's language "a fluid focusing region configured to focus the sample stream." (Appx28.) In the Board's view, the phrase "the sample stream" must be construed as "singular." (Appx28.) By "singular," the Board meant "one stream" that is not "split." (Appx27–28.) In other words, the Board interpreted "the sample stream" to require one single, contiguous stream.

Citing Figures 3(a) and 3(b) in Simonnet, the Board determined that Simonnet does not meet the requirement of having "the sample stream" as the Board construed the term, because the High-Throughput Device produces "split sample streams" with a "gap" in the middle. (Appx37.) Accordingly, based on its construction of "the sample stream," the Board held that Simonnet failed to anticipate claims 1, 2, 6, and 8. (Appx39; Appx48.) The Board rejected ABS's obviousness challenges on the same ground. (Appx55; Appx57; Appx59.)

ABS timely appealed the Board's decision. (Appx809–813.)

SUMMARY OF ARGUMENT

The Board's decision should be reversed, or at a minimum, vacated and remanded.

The Board's sole ground for finding the challenged claims patentable was an erroneous construction of the phrase "the sample stream" in claim 1. Claim 1 calls for "an inlet configured to receive a sample stream; [and] a fluid focusing region configured to focus the sample stream." (Appx95(18:48–50).) The Board incorrectly construed "the sample stream" to require one single, contiguous stream.

The phrase "the sample stream" cannot support the Board's construction, because "the sample stream" refers back to the earlier phrase "a sample stream," and the patent expressly defines "a" as used with an "entity" (such as "a sample stream") to mean "one or more of that entity." (Appx95(18:27–30).) Together, the "inlet" and "fluid focusing region" limitations call for a device configured to allow "a sample stream" to be received in the inlet, and then focused in the fluid focusing region. The phrase "*the* sample stream," like the phrase "*said* sample stream," does not communicate *how many* sample streams, but rather makes clear that *whichever* sample stream or streams enter the

inlet must then proceed to be focused in the fluid focusing region. Inferring singularity from this type of phrase was error. *See Rehco LLC v. Spin Master, Ltd.*, 759 F. App'x 944, 949–50 (Fed. Cir. 2019); *Baldwin Graphic Sys., Inc. v. Siebert, Inc.*, 512 F.3d 1338, 1342–43 (Fed. Cir. 2008).

The Board reached its erroneous construction in part based on a mistaken interpretation of the phrase "a centerline of the sample stream" in claim 2. Claim 2 requires that the lateral fluid focusing feature be "configured to introduce focusing fluid into the flow channel symmetrically with respect to *a centerline of the sample stream*." (Appx95(18:65–67) (emphasis added).) The Board concluded that "a centerline of the sample stream" is a line drawn through the middle of a sample that touches sample fluid, not focusing fluid, and further determined that such a centerline cannot be drawn through a split sample. To avoid this perceived problem, the Board held that "the sample stream" must be one single, contiguous stream.

The Board's reasoning was flawed for several reasons. As an initial matter, the Board misunderstood the nature of the "centerline" at issue in claim 2. Claim 2 discloses a "centerline of the sample

stream" for purposes of introducing focusing fluid "symmetrically," *i.e.*, evenly on both sides of the line. Achieving that symmetry has nothing to do with what substance the centerline touches; all that is required is that the line run through the middle of the sample, so that the same amount of focusing fluid can be introduced to the right of the line and to the left of it. A "centerline of the sample stream," therefore, can be drawn regardless of whether a sample has a gap in the middle. And in any event, even under the Board's construction of "a centerline of the sample stream," a split sample still has a requisite centerline, drawn down either part (or both parts) of the split sample. Thus, there was no reason to require one single, contiguous sample stream.

More fundamentally, the Board erred because it determined that "the sample stream" must be contiguous, rather than split, but as Simonnet and other prior art references make clear, a split sample is a product of how a device is used—and more specifically, the particular flow conditions created within a device's flow channel. The challenged patent claims, however, are device claims, not method-of-use claims. They impose no limitations on the flow conditions that would be necessary to produce a split sample or a contiguous sample. By

imposing a use-based limitation on device claims, the Board erred. *See Paragon Sols., LLC v. Timex Corp.*, 566 F.3d 1075, 1090–91 (Fed. Cir. 2009). The Board's erroneous claim construction requires, at a minimum, vacatur and remand.

However, rather than vacating the Board's decision, this Court should reverse because the challenged claims are unpatentable under any construction of "the sample stream." *See Owens Corning v. Fast Felt Corp.*, 873 F.3d 896, 901 (Fed. Cir. 2017). On a proper construction of claim 1, Simonnet disclosed a device configured to focus "the sample stream." Because "the sample stream" refers back to the plural term "a sample stream," it does not matter whether the stream has a gap in the middle, or whether a split stream is characterized as one stream or two.

Even under the Board's construction of "the sample stream" as one single, contiguous stream, Simonnet renders claim 1 unpatentable. Under a variety of flow conditions, the Simonnet device can focus a single, contiguous sample stream. Simonnet expressly teaches how skilled artisans can adjust the relative flow conditions to avoid any gap in the sample stream; and in any event, these adjustments would have been obvious to skilled artisans. In addition, even assuming the

Simonnet device invariably produces a split sample (which it does not), it still satisfies a "singular" stream requirement because a split sample is a single stream, just with a gap in the middle, as both Simonnet and other prior art references recognize.

Therefore, reversal is warranted because, whether this Court corrects the Board's construction of "the sample stream" or not, every challenged claim of the '439 patent is unpatentable.

ARGUMENT

I. Standard of Review

This Court reviews the Board's conclusions of law, including its "ultimate interpretation of patent claims," *de novo*, and its factual determinations for substantial evidence. *Unwired Planet, LLC v. Google, Inc.*, 841 F.3d 995, 1000 (Fed. Cir. 2016); *CardSoft, (assignment for the Benefit of Creditors), LLC v. VeriFone, Inc.*, 807 F.3d 1346, 1349 (Fed. Cir. 2015).

II. The Board Erred by Construing the Phrase "The Sample Stream" in Claim 1 to Require One Single, Contiguous Sample Stream.

The Board's decision turned on its erroneous construction of the phrase "the sample stream" in claim 1.

A. Contrary to the Board's Construction, "The Sample Stream" Refers Back to the Phrase "A Sample Stream," and Takes the Same Plural Meaning.

The phrase "the sample stream" in claim 1 draws meaning from its antecedent, "a sample stream." Read in isolation, the "fluid focusing region" limitation discloses "a fluid focusing region configured to focus the sample stream." (Appx95(18:49–50).) But from this language alone, it is unclear *which* sample stream is being discussed; no context is given to explain where "the sample stream" came from, or what its properties are, leaving its meaning uncertain.

That meaning becomes clear, however, in light of the immediately preceding limitation: "an inlet configured to receive *a sample stream*." (Appx95(18:48) (emphasis added).) This language establishes which "sample stream" is being discussed throughout the rest of the claim, avoiding potential vagueness problems. *See* U.S. Patent & Trademark Offices, Manual of Patent Examining Procedure § 2173.05(e) (9th ed. June 2020) (noting that a "lack of clarity could arise where a claim refers to 'said lever' or 'the lever,' where the claim contains no earlier recitation or limitation of a lever and where it would be unclear as to what element the limitation was making reference"); *Warner-Lambert*

Co. v. Apotex Corp., 316 F.3d 1348, 1356 (Fed. Cir. 2003) ("The words 'the use' require antecedent basis; thus, 'the use' refers to a specific 'use' rather than a previously undefined 'use.'"). Taken together, the two limitations provide that "a sample stream" enters the flow channel through an "inlet," and then exactly that which entered through the inlet—"a sample stream"—proceeds to be focused in the "fluid focusing region." (Appx95(18:48–50).)

When used in this way to "refer back to an initial indefinite article," phrases like "the sample stream" are known as "anaphoric phrases"—*i.e.*, phrases whose meaning depends upon a prior term or expression. *Baldwin Graphic Sys.*, 512 F.3d at 1343. In such phrases, the word "the" functions like the word "said." *Id.* at 1342–43; *see Hytera Commc'ns Co. v. Motorola Sols., Inc.*, 841 F. App'x 210, 218 (Fed. Cir. 2021) ("[E]ach step of the method provides an antecedent basis for the steps that follow. That includes 'a' timeslot in the 'preparing' step, which grammatically provides antecedent basis for 'the' timeslot in the 'determining' step.").

Like the word "said," the word "the" in "the sample stream" is not necessarily singular—indeed, it is not used to specify "how many"

streams there are at all. Rather, the word "the" in the phrase "the sample stream" imports the singular or plural nature of its antecedent. *See 01 Communique Lab., Inc. v. LogMeIn, Inc.*, 687 F.3d 1292, 1297 (Fed. Cir. 2012) ("The subsequent use of definite articles 'the' or 'said' in a claim to refer back to the same [non-singular] claim term . . . simply reinvokes that non-singular meaning." (citation omitted)).

Here, "the sample stream" adopts the plural meaning of its antecedent "a sample stream." Consistent with the phrase's plain meaning, the patent makes clear that "a" sample stream means "one or more" sample streams. (Appx95(18:27–30).) The phrase "the sample stream" simply "reinvokes that non-singular meaning." *01 Communique Lab.*, 687 F.3d at 1297 (citation omitted).

Therefore, as used in claim 1, neither "a sample stream" nor "the sample stream" is compatible with a requirement of one single, contiguous stream. The Board was wrong to hold that "the sample stream" refers to "a singular stream," and that this limitation cannot be satisfied "when there is more than one stream (i.e., in the case of a split sample)." (Appx28.)

This Court has previously vacated a decision that erroneously treated an anaphoric phrase using the word "the" as singular, rather than deriving its meaning from its antecedent phrase. In *Rehco*, for instance, this Court interpreted a patent for a remote-control vehicle that used the word "the" in the same way as claim 1 of the '439 patent. The patent in *Rehco* claimed:

> 1. A vehicle . . . comprising:
>
> a transmitter positioned on the bottom of said vehicle for transmitting *a signal* . . .
>
> a receiver positioned on the bottom of said vehicle for receiving *said signal* as it is bounced off of a surface . . . and
>
> a control system . . . having a first means to set the speed of the propelling means to a first speed when the receiver receives *the bounced signal* . . . .

759 F. App'x at 946 (second and third emphases added).

The district court in *Rehco* erroneously "construed 'a signal' to mean 'a single signal being emitted from the transmitter, and not multiple signals.'" *Id.* at 949. This Court explained that, as a rule, "a" or "an" means "one or more"; thus, "a signal" meant "one or more signals." *Id.* This Court emphasized that "[t]he subsequent use of definite articles 'the' or 'said' in a claim to refer back to the same claim

term does not change the general plural rule, but simply reinvokes that non-singular meaning." *Id.* (quoting *01 Communique*, 687 F.3d at 1297). Thus, the use of phrases like "the bounced signal" was "irrelevant" to determining how many bounced signals there were. *Id.* at 950. Rather, these phrases were merely stand-ins for the phrase "a signal"; because "a" signal meant "one or more" signals, so too did "the bounced signal." *Id.*

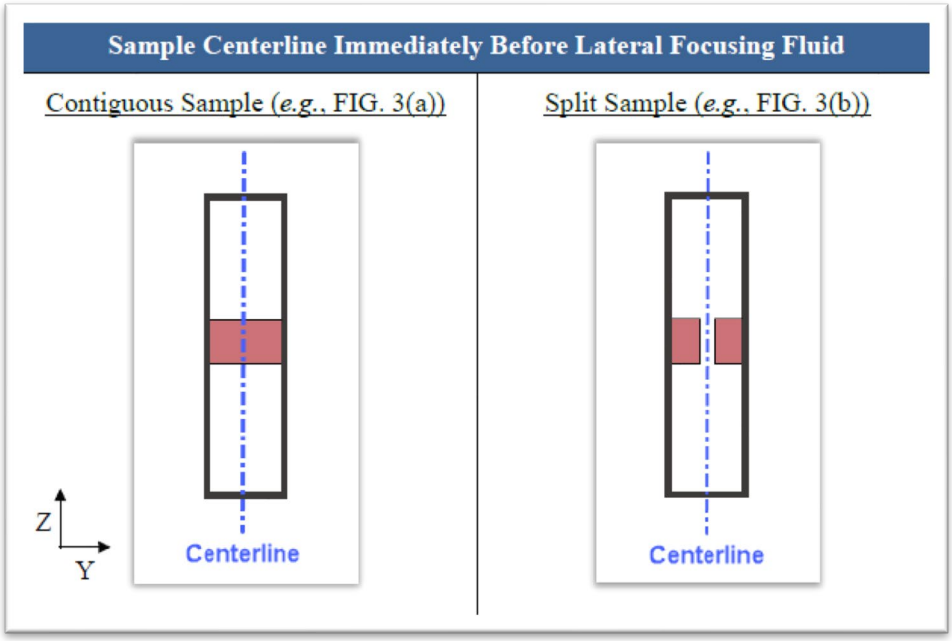
The same reasoning applies here. The phrase "the sample stream" functions as a stand-in for the antecedent phrase "a sample stream." Thus, "the sample stream" simply "reinvokes" the meaning of "a sample stream," *id.*, which the patent defines to mean "one or more" sample streams. (Appx95(18:27–30).) The phrase "the sample stream" "do[es] not alter that meaning in the slightest." *Baldwin Graphic Sys.*, 512 F.3d at 1343.

The Board noted that it is "presumed" that "a" or "an" means "one or more," but this understates this Court's precedent. (Appx23.) "That 'a' or 'an' can mean 'one or more' is best described as a rule, rather than merely as a presumption or even a convention." *Baldwin Graphic Sys.*, 512 F.3d at 1342. "The exceptions to this rule are extremely limited: a

patentee must evince a clear intent to limit 'a' or 'an' to 'one.'" *Id.* (internal quotation marks and brackets omitted) (citation omitted). The '439 patent does the opposite, expressly confirming that "the term 'a' or 'an' entity refers to one or more of that entity." (Appx95(18:27–28).) That meaning applies to "a sample stream," and by extension, "the sample stream."

B. The Board's Erroneous Construction Was Based in Part on a Mistaken Interpretation of Claim 2's Phrase "A Centerline of the Sample Stream."

Beyond misinterpreting the word "the" in "the sample stream," the Board's construction requiring one single, contiguous stream was based in part on the phrase "a centerline of the sample stream" in claim 2. Claim 2 adds a requirement to claim 1 that "the lateral fluid focusing feature is configured to introduce focusing fluid into the flow channel symmetrically with respect to *a centerline of the sample stream*." (Appx95(18:64–67) (emphasis added).) In the Board's view, a "centerline of the sample stream" refers to a line drawn through the middle of a sample that "runs through" sample fluid, rather than focusing fluid, as shown in the example of a "contiguous sample" on the left side of the figure below. (Appx24–25.)



(Appx24 (depicting sample in red).)

A line drawn through the middle of a sample that "runs through sheath fluid," as shown on the right side of the figure above, would not qualify as "a centerline of the sample stream," in the Board's view. (Appx25.) Based on this view, the Board reasoned that "it might not be possible to draw a centerline of a sample stream" running through sample fluid "when there is more than one stream (i.e., in the case of a split sample)." (Appx27–28.) Thus, the Board concluded that "the sample stream" must be one "singular" stream. (Appx28.)

The Board's reasoning does not support its construction of "the sample stream." First, the Board's interpretation of "a centerline of the

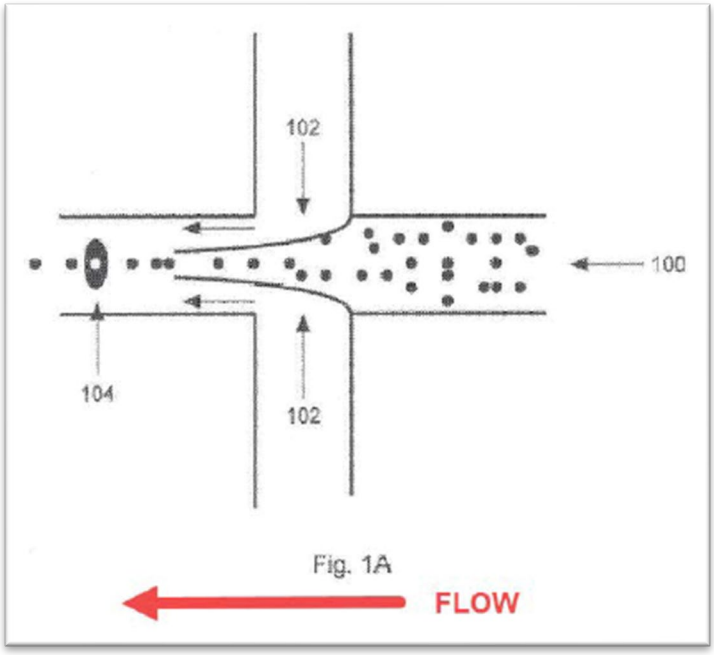
sample stream" is incorrect because the Board improperly defined the term by reference to what substance the "centerline" touches, which has no basis in the relevant claim language. Second, even under the Board's interpretation of a "centerline," it is possible to draw such a centerline without having to require one single, contiguous sample stream. The Board thus created a limitation to solve a problem that did not exist.

1. The Board Failed to Recognize that a "Centerline" Is the Middle of the Sample for Purposes of Introducing Focusing Fluid Equally on Both Sides.

First, the Board erred by defining a "centerline of the sample stream" in terms of what substance the centerline touches. Claim 2 does not define a centerline by reference to whether that line "runs through" sample fluid or focusing fluid. Rather, claim 2 defines a centerline for purposes of creating symmetry with respect to the introduction of focusing fluid from the right and left of the sample. (*See* Appx95(18:65–67) (providing that the lateral fluid focusing feature must be "*configured to introduce focusing fluid into the flow channel symmetrically* with respect to a centerline of the sample stream") (emphasis added).) The centerline, therefore, simply identifies the

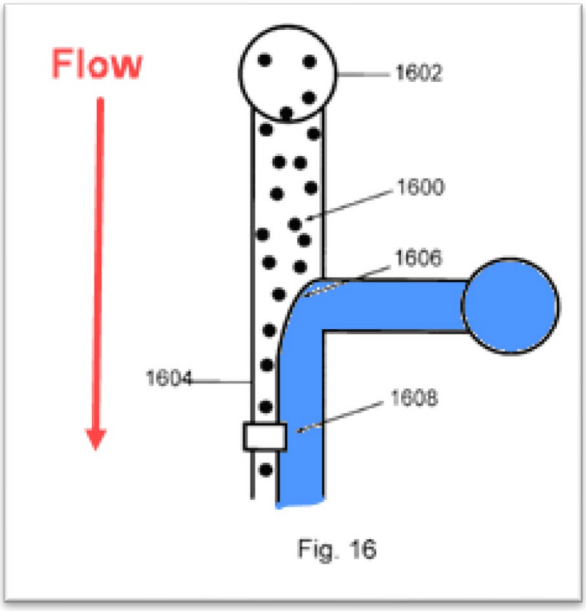
middle of the sample—regardless of what shape the sample flow might take—so that equal amounts of focusing fluid can be introduced to the right of the line and to the left of it. (*See* Appx4181–4185; *see also* Appx4358 (Cytonome's expert stating that claim 2's symmetry requires "determin[ing] whether the sheath fluid is introduced equally on either side of the centerline").)

This type of symmetry is often utilized in the field of microfluidics. (*See* Appx4183–4185 ("[S]ymmetrical introduction of sheath fluid (*i.e.*, equal amounts from both sides simultaneously) was conventional and reflected in numerous prior art references.") (depicting three examples); *see also, e.g.*, Appx2078.) Among other things, symmetrically introducing focusing fluid can improve a user's ability to evaluate cells one by one by squeezing them into a row in the middle of the device's flow channel. (*See, e.g.*, Appx2117 (explaining that focusing particles "near the middle of the chamber cross section" can facilitate "a well-defined passage time through the illumination spot" in the inspection region).) Such symmetry is shown in the example below, where equal amounts of sheath fluid (102) are introduced from either side of the sample (100) as it flows from right to left through a device:



(Appx1986 (annotating Appx2199(fig. 1A)).)

The alternative to this kind of symmetrical introduction of focusing fluid is to introduce focusing fluid asymmetrically, as shown in an example discussed earlier and reproduced below:



(Appx4159–4160 (annotating Appx2213(fig. 16)).) Here, focusing fluid (in blue) is introduced from just the right side, focusing the sample to the left. (*See* Appx4159–4160.)

The Board's concern about whether the centerline touches focusing fluid or sample fluid is irrelevant to claim 2's limitation. In order to create the required symmetry (*i.e.*, by balancing the focusing fluid simultaneously introduced from both sides of a sample), the middle of the sample provides the proper point of reference, regardless of whether the middle contains sample fluid, focusing fluid, or both. (Appx4181; *see* Appx2121 (Simonnet describing the gap in sample fluid as occurring "*in the middle*" of the sample stream) (emphasis added).) Nothing in claim 2 distinguishes between a split sample and a contiguous sample, or requires that "the sample stream" be one contiguous stream.

The Board reasoned that "a centerline of the sample stream" must touch sample fluid to avoid being redundant with phrases like "a centerline of the flow channel." (Appx24–25.) The Board noted that other claims in the '439 patent use phrases like "a centerline of the flow channel" or "a centerline of the microfluidic channel," and concluded

that these phrases must be distinguished by holding that "a centerline of the sample stream" cannot "run[] through sheath fluid." (Appx25.)

The Board perceived a problem where none existed, however. When properly construed in terms of the symmetrical introduction of focusing fluid, "a centerline of the sample stream" is still distinct from phrases like "a centerline of the flow channel." In particular, "a centerline of the sample stream" is a limitation related to the *flow conditions* that may be created when using a device, whereas phrases like "a centerline of the flow channel" place limitations on the *physical features* of the device, regardless of whether fluid is flowing through it at all.

For instance, claim 5 discloses a requirement that two fluid focusing channels "are symmetrically arranged with respect to *a centerline of the flow channel*." (Appx96(19:23–25) (emphasis added).) This limitation affects the physical features of the device only: To meet claim 5's requirement, the fluid focusing channels must be placed across from one another, evenly spaced from the middle of the flow channel. The symmetry described in claim 5 has nothing to do with focusing fluid or the flow conditions created by that fluid; because only the physical

features of the device are at issue, the middle of the "flow channel" provides the proper point of reference.

Claim 2, in contrast, requires that the lateral fluid focusing feature be "configured to introduce *focusing fluid*" symmetrically on both sides of the sample. (Appx95(18:65–67) (emphasis added).) This type of symmetry turns on more than just the placement of the physical features of the device. For example, "symmetrical introduction" of sheath fluid requires "*equal amounts* from both sides simultaneously," (Appx4183 (emphasis added)), so even if the focusing features themselves were symmetrically arrayed—*e.g.*, identical inlets on either side of the main flow channel—the "introduction" of focusing fluid would not be symmetrical if the device were configured to introduce more sheath fluid from one inlet than the other. (*See, e.g.*, Appx2237(34:27–32) (claiming a method that "introduc[es] a fluid flow" from "only one of the two opposing microchannels"); Appx4004–4006 (describing an example where opposing "side channels introduce fluid continuously, but their *relative flow rates are adjusted*," pushing the sample in one direction or another) (emphasis added).) Thus, to satisfy the symmetry required by claim 2, the "centerline of the sample

stream," rather than the "centerline of the flow channel," provides the proper point of reference.

Because the phrase "a centerline of the sample stream" already has a distinct meaning from other "centerlines" discussed in the patent, there was no need for the Board to distinguish between them by creating a new limitation requiring one single, contiguous sample stream.

2. Even Under the Board's Mistaken Approach, Every Sample Stream Has a "Centerline."

The Board's "single, contiguous stream" requirement was also improper because, even under the Board's mistaken approach to the phrase "a centerline of the sample stream," every sample stream has a centerline running through sample fluid as the sample stream enters the inlet to the flow channel. The Board recognized that "the sample stream" in the phrase "a centerline of the sample stream" "refers back, as a matter of antecedent basis, to 'a sample stream' in claim 1, i.e., 'an inlet configured to receive a sample stream.'" (Appx25–26 (quoting Appx95(18:48)).) Based on this, the Board considered the possibility that "a centerline of the sample stream" is a line drawn through the middle of the sample upon its entry into the inlet. (Appx26.) At the

point of entry into the inlet, no splitting is possible because the flow channel has not yet reached a vertical fluid focusing feature that might cause a split. Therefore, "a centerline of the sample stream" can always be drawn through sample fluid, satisfying the Board's construction.

The Board rejected this reasoning, however, by overreading purely relative terms like "upstream" and "downstream." The Board noted, for instance, that claim 1 imposes a requirement that "a bottom surface of the flow channel lies in a first plane *upstream* of the first and second vertical fluid focusing features." (Appx26; Appx95(18:55–58) (emphasis added).) From these phrases, the Board concluded that the term "stream" must "refer to the entire stream that flows through the device, and not simply the portion of the stream that enters the device." (Appx26.)

The Board erred because words like "upstream" are relative, location-based terms that do not create limitations on the shape or flow conditions of "the sample stream." The word "upstream" uses the word "stream" to identify a location *based on the direction a sample stream would flow through the flow channel*. For instance, claim 1 requires that "a bottom surface of the flow channel lies in a first plane" in a

particular location along the flow channel: "*upstream* of the first and second vertical fluid focusing features." (Appx95(18:55–58) (emphasis added).) In other words, because the flow of the sample stream starts at the "inlet configured to receive a sample stream," and then goes down through the "fluid focusing region," and finally to the "inspection region," the phrase "upstream of the first and second vertical fluid focusing features" refers to an area between the inlet and the first and second vertical fluid focusing features. Terms like "upstream" have nothing to do with whether "a centerline of the sample stream" can be drawn when "a sample stream" enters the inlet to the flow channel. In all cases, it can.

In any event, even assuming that a "centerline of the sample stream" must run through sample fluid as the sample stream flows through the device, every sample stream still has such a centerline. Even if a sample stream is split by focusing fluid, one can draw a centerline running through sample fluid in two places—*i.e.*, down the middle of either part of the split sample (or both parts). With respect to either of these centerlines, the lateral fluid focusing feature could be

configured to introduce focusing fluid "symmetrically," *i.e.*, in equal amounts on either side of the line. (Appx4182.)

The Board acknowledged the possibility of each part of a split stream "having its own centerline," but concluded that "it would not be possible to 'introduce fluid into the flow channel symmetrically with respect to a centerline of the sample stream,' as claimed in claim 2." (Appx27.) That is incorrect. For either centerline drawn through either part of the split sample, focusing fluid can be introduced "equally on either side of the centerline." (Appx4358 (Cytonome's expert describing the symmetry of claim 2).) In other words, the same amount of focusing fluid can be injected on the right side of the line as on the left side. (*See* Appx4182 ("Even if 'a centerline' were improperly drawn through one portion of the split sample, the lateral fluid focusing feature would still simultaneously introduce equal amounts of focusing fluid from either side of the centerline . . . .").) It was undisputed before the Board that the Simonnet device is configured to have such balanced introduction of focusing fluid, and that is all that claim 2's limitation requires.

Accordingly, nothing in claim 2's language "a centerline of the sample stream" justified the Board's requirement of one single, contiguous stream.

C. The Board Improperly Imposed a Use-Based Limitation on a Device Claim.

In addition to the flaws in the Board's reasoning discussed above, the Board's requirement of one single, contiguous stream was erroneous because it was improperly directed at a particular way of *using* the claimed device. The Board determined that "the sample stream" must be contiguous, rather than split (Appx23; Appx28), but as those in the field widely recognized, a split sample occurs only if a device is used in a way that creates specific relative flow conditions. (*See, e.g.*, Appx2782 (noting the presence of a "gap in the middle" of "the focused stream," and explaining that "[t]his irregular shape is due to the fact that the flow rate injected from inlet A (sheath flow) is 12 times higher than that injected from inlet B (sample flow) and the finite aspect ratio of the main channel (A channel)"); Appx2832; Appx3799.) In other words, the emergence of a split in a sample stream is a product of how a microfluidic device is used, not an inherent part of its design.

The challenged claims, including claims 1 and 2, say nothing about the flow conditions that would produce a split sample, because they are device claims, not method-of-use claims. (Appx22 n.13 (Board noting that claim 1 is "silent as to changes in flow rate"); *see also, e.g.*, Appx95(18:65) (addressing how the lateral fluid focusing feature must be "configured").) Patent claims may be directed to either a device or a method of use, but they cannot cover both. *See IPXL Holdings, L.L.C. v. Amazon.com, Inc.*, 430 F.3d 1377, 1384 (Fed. Cir. 2005) (invalidating a patent claim as indefinite because it covered "both a system and the method for using that system"). Thus, a device claim cannot be interpreted so as to create use-based limitations. *See Paragon Sols.*, 566 F.3d at 1090 (rejecting a construction that "injects a use limitation into a claim written in structural terms," because "[a]pparatus claims cover what a device is, not what a device does" (citation omitted)).

The Board's requirement of one single, contiguous sample stream was precisely that: a use-based limitation imposed on device claims. The Board interpreted "the sample stream" in order to rule out particular uses of the patented device—specifically, those that would create a split sample. This conflation of features of the device and

methods of using the device only confused the challenged claims. *See Paragon Sols.*, 566 F.3d at 1091 ("Construing a non-functional term in an apparatus claim in a way that makes direct infringement turn on the use to which an accused apparatus is later put confuses, rather than clarifies, frustrates the ability of both the patentee and potential infringers to ascertain the propriety of particular activities, and is inconsistent with the notice function central to the patent system.").

The challenged claims speak only to how the "microfluidic assembly" must be "configured." (*See, e.g.*, Appx95(18:48–50).) As long as the device is constructed to have the necessary capabilities, the claim limitations are satisfied. *See Finjan, Inc. v. Secure Computing Corp.*, 626 F.3d 1197, 1204 (Fed. Cir. 2010) ("[T]o infringe a claim that recites capability and not actual operation, an accused device need only be capable of operating in the described mode." (internal quotation marks omitted) (citation omitted)).[8] And as long as a prior art reference

[8] Conversely, if the '439 Patent were permitted to incorporate the Board's use-based limitation, determining whether any other focusing device infringed on the patent claims would turn in part on whether the device was being used at the time to produce a split stream. Thus, an otherwise *identical* device might or might not infringe the patent claims, depending on its usage.

disclosed the same capabilities, the challenged claims are unpatentable. *See ParkerVision, Inc. v. Qualcomm Inc.*, 903 F.3d 1354, 1361 (Fed. Cir. 2018) ("[A] prior art reference may anticipate or render obvious an apparatus claim . . . if the reference discloses an apparatus that is reasonably capable of operating so as to meet the claim limitations, even if it does not meet the claim limitations in all modes of operation.").

The Board's introduction of a use-based requirement that went beyond the "configuration" of the device was a fundamental error. Because that erroneous construction formed the sole basis for the Board's ruling on all of the challenged claims, the Board's decision should, at a minimum, be vacated and remanded.

III. Reversal Is Warranted Because the Only Conclusion Supported by the Evidence Is That the Challenged Claims Are Unpatentable.

Instead of vacating and remanding, however, this Court should reverse the Board's determination because the challenged claims are unpatentable under any available construction of the term "the sample stream." *See Owens Corning*, 873 F.3d at 901 (holding that it was "not necessary or appropriate to remand for the Board to reassess the

evidence in light of the correct claim construction" because the substantial evidence supported only the conclusion that the challenged claims were invalid).

A. Under Any Construction of "The Sample Stream," Simonnet Anticipates "The Sample Stream" Limitation in Claim 1.

The Board rejected ABS's anticipation challenge solely on the ground that "Simonnet's split sample does not satisfy 'the sample stream' limitation" in claim 1. (Appx39; *see* Appx48 (applying the same reasoning to the dependent claims).) The Board's decision must be reversed because Simonnet satisfies "the sample stream" limitation under any available construction of that term.

As discussed above, the proper construction of the phrase "the sample stream" is a reference back to the plural term "a sample stream." Under this construction, Simonnet anticipates claim 1 because the High-Throughput Device comprises a fluid focusing region that is configured to focus—*i.e.*, "pinch[], confin[e], squeez[e], or constrict[]" (Appx28)—"the sample stream." Simonnet is "literally a 'textbook

design"'[9] of a device that focuses sample fluids. (Appx1995; *see, e.g.*, Appx2959 (noting that Simonnet has been cited hundreds of times by other skilled artisans).) Because "the sample stream" simply refers to "a sample stream," it does not matter whether one treats a split sample as two different sample streams or one stream with a gap in the middle. (*Compare* Appx37 (Board concluding that "Simonnet discloses split sample *streams*" (emphasis added)), *with* Appx5657 (Simonnet describing a gap "in the middle" of "*the stream* from inlet B" (emphasis added)).) However a split sample is characterized, the High-Throughput Device is configured to focus that "sample stream." (*See* Appx2121 ("The stream from inlet B . . . is confined to regions with y–z cross sections of 5 x 10 and 5 x 4 μm.").)

Even on the Board's construction of "the sample stream," moreover, Simonnet anticipates claim 1 for two reasons.

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[9] (*See* Appx2531–2533 (textbook depicting the High-Throughput Device and explaining that "[t]he sample stream was focused to approximately 10 μm and an impressive 17,000 count/s were achieved with this device, rivaling commercial nonmicrofabricated flow cytometers"); Appx3923–3924 (noting that, using the High-Throughput Device, "the size, aspect ratio, and position of the sample stream can be controlled"); Appx3965–3967.)

First, the High-Throughput Device is "configured to focus" one single, contiguous stream. Simonnet is explicit that a gap in the sample stream "occurs" only if the device is used to produce certain relative flow conditions. (Appx2121; *cf.* Appx2124 (noting for another device that a gap "occurs when the rate of flow injected from channels B is small (as compared with the rate of flow in channel A)").) Under a wide range of other flow conditions, no gap will appear. (*See* Appx2121; Appx2126 (noting that the "cross section of the stream can be adjusted"); Appx4171–4173; *cf. also* Appx2782(fig. 7(a)) (depicting in a different device how the gap in the sample stream disappears as flow conditions are adjusted).) That is all that is necessary for the High-Throughput Device to be adequately "configured." *See ParkerVision, Inc.*, 903 F.3d at 1361 ("[A] prior art reference may anticipate or render obvious an apparatus claim . . . if the reference discloses an apparatus that is reasonably capable of operating so as to meet the claim limitations, even if it does not meet the claim limitations in all modes of operation.").

The Board never addressed the evidence that the Simonnet device can produce a single, contiguous stream under a variety of flow

conditions. Instead, the Board concluded that "Simonnet discloses split sample streams" based on the figure below, stating that "the stream of Figure 3(b) is 'split into two parts, with a gap in the middle' and '[a] residual gap can also be seen in Figure 3a.'" (Appx37 (quoting Appx2121).)

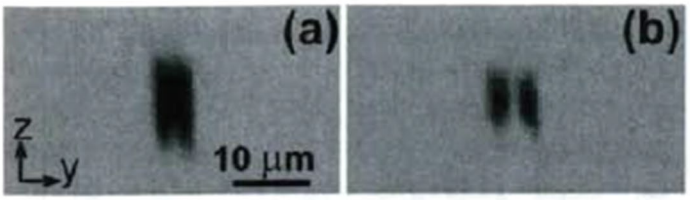


Figure 3. Confocal micrographs showing flow structure near the central axis of the cytometry channel in the HTD at conditions typical for cytometry assays. Fluorescent dye (FITCD) was injected into ports A, C, and E. Dark areas correspond to the stream from port B. Differential pressures at ports A, B, C, and E in kPa with respect to port F were: (a) 9.5, 8.9, 9.2, and 9.1; and (b) 9.5, 8.6, 9.2, and 10.2.

(Appx2121(fig. 3).)

Those figures, however, only depict two possible sets of flow conditions. (Appx2121 (noting that figure 3 depicts "[t]wo profiles of fluorescence at flow conditions typical for the cytometry assays").) These two examples, moreover, reinforce Simonnet's teaching that a split sample occurs only under certain conditions. Unlike the obvious gap depicted in Figure 3(b), Figure 3(a) reflects an apparently contiguous sample stream, save for a notch at the bottom. Simonnet

describes this as a "residual gap." (Appx2121.) The difference is the flow conditions; the pressures (which drive the relative flow rates of the various fluids) are described in the caption to Figure 3. (Appx2121; *see also* Appx4175–4178.) ABS submits that the Board clearly erred in concluding that Figure 3(a)'s "residual gap" is a split stream at all—as opposed to the "residue" of a gap that has been *eliminated*—but if the flow conditions were adjusted even further, any asserted gap in Figure 3(a) would disappear entirely. (*See* Appx2121 (stating that a "gap occurs" only under certain flow conditions).)

The Board's only other basis for concluding that Simonnet does not anticipate claim 1 was its rejection of an argument that ABS never made. The Board concluded that ABS failed to show "that Simonnet would incorporate an embodiment from" an earlier work, referred to as "Simonnet 2005," that also discussed adjusting flow conditions to avoid a split sample. (Appx39.) But ABS never argued "incorporation-by-reference" in its briefing to the Board. (*See* Appx598 n.3 (Cytonome acknowledging that "Petitioners do not expressly assert that Simonnet incorporates Simonnet 2005 by reference").) Rather, ABS argued that "Simonnet alone" teaches that the High-Throughput Device is

configured to produce a singular sample stream "under a wide range of conditions." (Appx530–531.) Because the Board failed to address ABS's actual anticipation argument or recognize that Simonnet itself disclosed a device configured to focus a single, contiguous sample stream, the Board's decision cannot stand. *See Cook Grp. Inc. v. Boston Sci. Scimed, Inc.*, 809 F. App'x 990, 997 (Fed. Cir. 2020) (finding error where the Board failed to address an anticipation argument).

Second, even if the High-Throughput Device were invariably to produce a split sample, Simonnet would still anticipate claim 1. The Board incorrectly assumed that a split sample constitutes two separate sample streams, and therefore cannot satisfy a requirement of a "singular" sample stream. (Appx49.) But Simonnet teaches that a sample stream that splits does *not* become two separate streams, but rather acquires a new feature: "a gap in the middle." (Appx2121.[10]) Thus, subsequent citations to Simonnet consistently recognized that the High-Throughput Device focuses a sample stream, without indicating

[10] Similarly, other prior art references addressing split samples have referred to the split as a change in "shape," rather than a new stream. (Appx2782; *see* Appx4164–4166.)

that it converts one stream into two separate streams. (*See* Appx3906 (explaining that Simonnet "focus[ed] a sample flow with three sheath inlets"); Appx2819 (noting that, in Simonnet, "the relative sheath flow values of the six inlets were used to fully control the position and size of the sample stream").)

The Board itself recognized that a sample stream can be "focused" even if the device also adds the separate feature of a gap in the middle. (Appx22 n.13 ("Patent Owner has not adequately explained why there could not be a narrowing of the sample overall ('focusing') when there is a split stream.").) Thus, whether the High-Throughput Device splits a sample stream or not is irrelevant to whether the device is "configured to focus" the sample stream. *See Boesen v. Garmin Int'l, Inc.*, 455 F. App'x 974, 977 (Fed. Cir. 2011) ("The district court correctly explained that as long as the Acura navigation system can—and does—perform the claimed input method, it is of no consequence that it can also perform other input methods."); *Orion IP, LLC v. Hyundai Motor Am.*, 605 F.3d 967, 977 (Fed. Cir. 2010); *Exergen Corp. v. Wal-Mart Stores, Inc.*, 575 F.3d 1312, 1319 (Fed. Cir. 2009). The Simonnet device is properly configured, as skilled artisans have long recognized.

Therefore, reversal is warranted because, under any construction of "the sample stream," Simonnet anticipates claim 1.

B. Even If Simonnet Does Not Anticipate Claim 1, Simonnet Renders Claim 1 Obvious.

Even if Simonnet does not fully anticipate claim 1, that claim is obvious from Simonnet alone, including based on Simonnet's disclosure of how a skilled artisan can adjust the relative flow rates within the High-Throughput Device to achieve the type of "singular" sample stream required by the Board. As discussed above, Simonnet explains that, by modifying the device's relative flow rates to prevent a situation in which "the flow emerging from channels B is small compared with the flow in channel A," a skilled artisan can avoid any "gap in the middle" of the sample stream. (Appx2121.) Thus, even if one assumes that Simonnet requires "modification," the only modification to the High-Throughput Device necessary to satisfy the Board's construction of "the sample stream" simply requires using "known methods"—indeed, adjustments to the relative flow conditions expressly taught in Simonnet itself—to "yield predictable results." *KSR Int'l Co. v. Teleflex, Inc.*, 550 U.S. 398, 416 (2007). (*See also* Appx500 (Cytonome acknowledging that "a [person of ordinary skill in the art] would have

followed the guidance within Simonnet itself and decreased the relative flow rate of the sheath fluid").)

The Board rejected ABS's obviousness challenge because, in the Board's view, ABS had "not provided adequate reasoning as to why a person of ordinary skill would have sought to modify the teachings of Simonnet, e.g., to vary the flow rate ratios and eliminate the gap in the sample streams." (Appx51; *see* Appx55 (citing the same reason for claims 2, 6, and 8).) In doing so, the Board "adopted an overly rigid obviousness analysis" that failed to "consider the knowledge and creativity of a skilled artisan." *C.R. Bard, Inc. v. Medline Indus., Inc.*, No. 2020-1900, 2021 WL 3574043, at *6 (Fed. Cir. Aug. 13, 2021).

In the field of microfluidics, it was well known that achieving a single, contiguous sample stream could be beneficial for certain applications. For instance, as Cytonome's expert explained, such a stream can "improve[] the precision with which the cell sample can be positioned in the observation region of the cytometer by restricting cells to the central region of the stream," while also "reduc[ing] the likelihood of obstruction of the flow system." (Appx4329 (quoting Appx3556).) Thus, a single, contiguous stream can "align particles into the center of

a channel for faster and more accurate processing of individual particles, such as inspecting particles in a flow cytometer." (Appx4330; *see* Appx2138 (discussing "[b]lood cell analysis by flow cytometry," which can "require[] hydrodynamic focusing . . . forcing the particles to pass the measuring sensor in single file").) For such applications, a split stream could be less desirable because the split may divert the sample stream "away from the center of the channel." (Appx4332.)

Indeed, Cytonome argued to the Board that a single, contiguous stream is *essential* to achieving proper focusing. (*See, e.g.*, Appx468 (arguing that "a 'split' sample stream is the antithesis of a 'focused' sample stream"); Appx477 (citing expert evidence to claim that "the advantages of focusing cannot be realized" in a split sample).) Cytonome's expert, for instance, contended that "[a] split sample stream . . . *invites* (and essentially guarantees) spatial coincidence of particles in each of the separate streams," thereby affecting the skilled artisan's ability to analyze or sort the particles. (Appx4240–4241; *see also* Appx4341 ("[P]ositioning of the sample at the center of the channel allows for accurate inspection of the sample, increases the flow velocity

of the sample core, and protects particles within the sample from high shear forces at the channel wall."); Appx4338–4357.)

Based on these motivations, skilled artisans, exercising "ordinary creativity," would naturally have made the adjustments to the relative flow conditions needed to cause the High-Throughput Device to produce a single, contiguous sample stream. *KSR Int'l Co.*, 550 U.S. at 421; *see id.* at 418 ("[A] court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ."); *C.R. Bard Inc.*, 2021 WL 3574043, at *7 (finding that skilled artisans would have been motivated to make obvious modifications "[g]iven the prior art disclosures and the finite number of predictable options"). As a result, claim 1 is unpatentable as obvious.

C. The Challenged Dependent Claims Are Also Unpatentable.

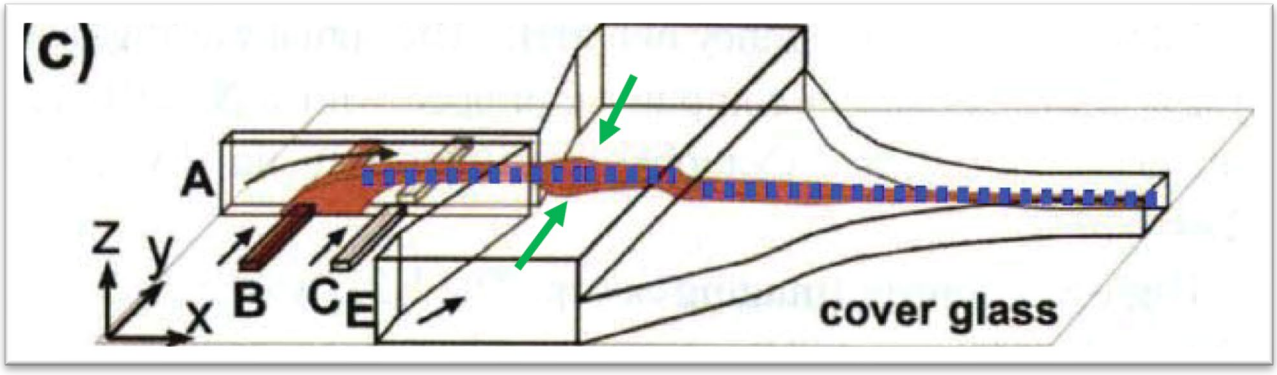
1. Simonnet Alone Anticipates or Renders Obvious Claims 2, 6, and 8.

The Board rejected ABS's challenges to dependent claims 2, 6, and 8 solely on the ground that Simonnet does not meet the "single, contiguous stream" requirement the Board read into claim 1. (Appx48.) Thus, if Simonnet anticipates claim 1—and it does, for all of the reasons

discussed above—then it likewise anticipates claims 2, 6, and 8. Similarly, if Simonnet alone renders obvious claim 1, then it likewise renders obvious claims 2, 6, and 8.

Claim 2. First, claim 2 discloses "[t]he microfluidic assembly of claim 1," with one additional element: "the lateral fluid focusing feature is configured to introduce focusing fluid into the flow channel symmetrically with respect to a centerline of the sample stream." (Appx95(18:64–67).) As discussed above, this symmetry is achieved by introducing "equal amounts" of focusing fluid "from both sides simultaneously." (Appx4183.)

Simonnet achieves the same symmetry through its lateral fluid focusing feature, which can introduce focusing fluid from the left and right sides of the sample stream simultaneously and in equal amounts, thereby "squeezing the stream" laterally. (Appx2120.) This type of symmetrical introduction of focusing fluid is depicted below.



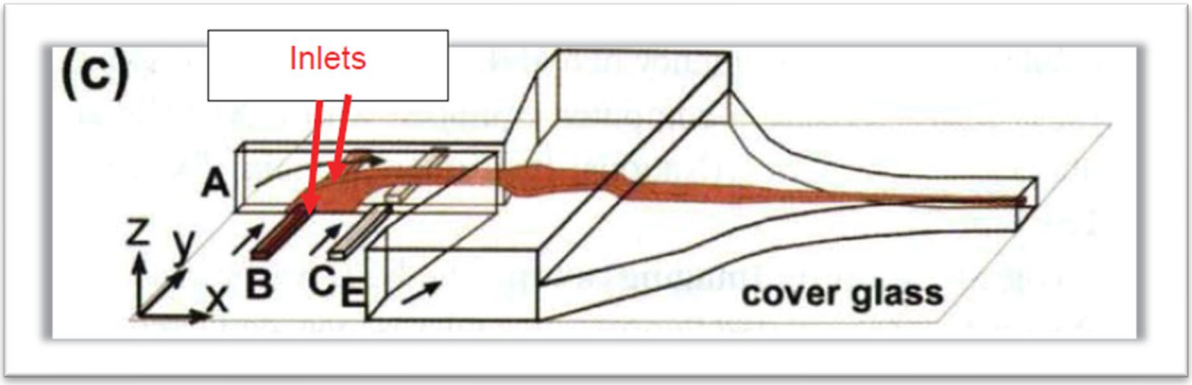
(Appx2078 (annotating Appx2119(fig. 1(c))).)

In the figure above, a blue, dotted centerline is drawn down the middle of the sample stream (depicted in red), as it flows through the High-Throughput Device. As the sample stream enters the wider portion of the fluid focusing region, Port E can introduce focusing fluid on both sides of the centerline, as depicted by the green arrows. Because Port E can introduce focusing fluid in equal amounts on either side of the centerline, *i.e.*, "symmetrically," Simonnet anticipates (or at a minimum, renders obvious) claim 2. (*See* Appx2078–2079; Appx4181–4185.)

Claim 6. Next, claim 6 discloses "[t]he microfluidic assembly of claim 1" with a different additional limitation: "the sample stream and the focusing fluid associated with the lateral fluid focusing feature enter the fluid focusing region in a same plane." (Appx96(19:26–29).) This

limitation requires that there be "a" plane, *i.e.*, one or more planes, in which both "the sample stream" and "the focusing fluid associated with the lateral fluid focusing feature" overlap in entering the fluid focusing region. (*See* Appx2082.)

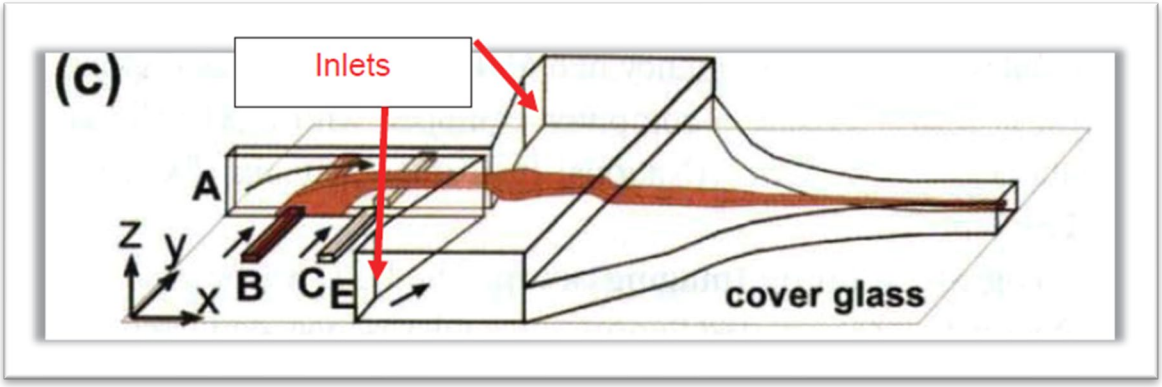
Simonnet teaches that the High-Throughput Device contains an infinite number of such overlapping planes. Simonnet explains that the sample fluid enters the flow channel through side channels (Port B) along the bottom of the chip, as shown below.



(Appx2080 (annotating Appx2119(fig. 1(c))).)

Upon reaching the flow channel, the sample fluid enters in an infinite number of horizontal planes between the bottom of the channel and 20 μm above the bottom of the channel. (Appx2080 (discussing Appx2119).)

Meanwhile, focusing fluid is introduced laterally through Port E, as shown below.



(Appx2081 (annotating Appx2119(fig. 1(c))).)

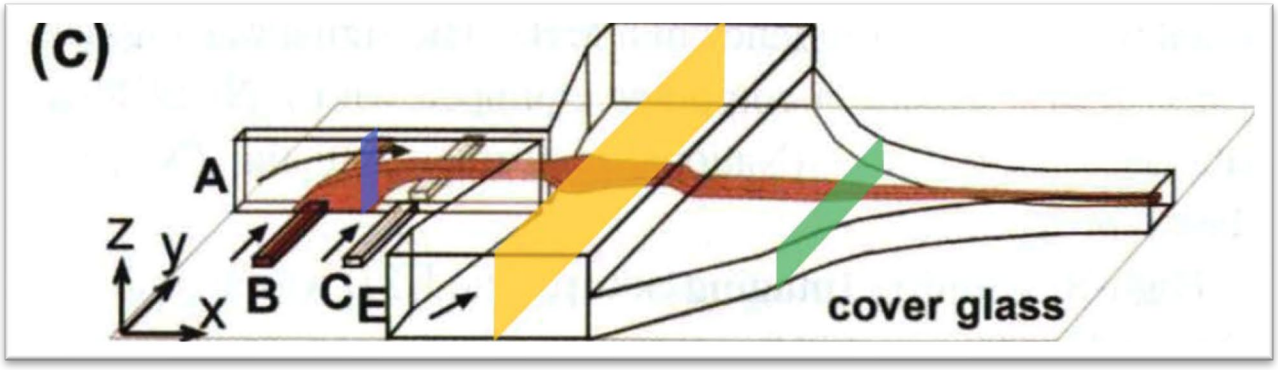
Simonnet provides that the focusing fluid enters the flow channel from Port E in an infinite number of planes between the bottom of the channel and 200 μm above the bottom of the channel. (Appx2081–2082 (discussing Appx2119).)

As a result, both the sample fluid and the focusing fluid associated with the lateral fluid focusing feature enter the fluid focusing region in an infinite number of planes between the bottom of the channel and 20 μm above the bottom of the channel (with the focusing fluid rising as high as 200 μm above the bottom of the channel). The fluids share overlapping planes throughout this 20-μm-tall region. Thus, Simonnet

anticipates (or at a minimum, renders obvious) claim 6. (*See* Appx2079–2082; *see also* Appx4185–4188.)

Claim 8. Finally, claim 8 discloses "[t]he microfluidic assembly of claim 1," adding that "within the fluid focusing region the fluid flow channel transitions from a first cross section shape to a second cross section shape different from the first cross section shape." (Appx96 (19:36–39).) In other words, the two-dimensional cross section of the flow channel changes from one shape to another, including different types of rectangles and squares. (*See* Appx89(5:62–67) ("The micro channel may have any selected cross-sectional shape or arrangement, non-limiting examples of which include a linear or non-linear configuration . . . and/or a rectangular, triangular, elliptical/oval, circular, square, or trapezoidal geometry.").)

It was undisputed below that Simonnet teaches this additional limitation. (Appx526 n.4 (ABS asserting that the limitation was undisputed; Appx594–613 (Cytonome not contesting the point).) In any event, the flow channel in the High-Throughput Device meets this requirement by changing shape multiple times, as shown below:



(Appx2085 (annotating Appx2119(fig. 1(c))).)

First, on the left side of the figure, the flow channel's cross section takes the shape of a tall, skinny rectangle (depicted in purple). Then, as the flow channel reaches the lateral fluid focusing feature, the flow channel's cross section broadens into a wide rectangle (depicted in orange). Finally, after the flow channel shifts down to a lower plane, its cross section narrows into a shorter and less wide rectangle (depicted in green). Thus, the flow channel "transitions" to a different "cross section shape" twice, anticipating (or at a minimum, rendering obvious) claim 8.[11] (*See* Appx2082–2086.)

[11] To the extent Simonnet alone does not fully anticipate or render obvious claim 8, claim 8 is obvious in light of Simonnet in combination with a reference referred to as "Kim." (Appx2786–2789; *see* Appx2086–2088 (explaining that "Kim teaches varying the width of the channel before and after each vertical fluid focusing feature," such that the cross section of the flow channel necessarily transitions from a rectangle to a square).)

* * *

As discussed above, Simonnet alone anticipates or renders obvious claim 1. And because claim 1 is unpatentable in view of Simonnet alone, claims 2, 6, and 8—which add limitations expressly taught by Simonnet—are unpatentable as anticipated or obvious, too.

2. Simonnet and Kummrow Render Claim 9 Obvious.

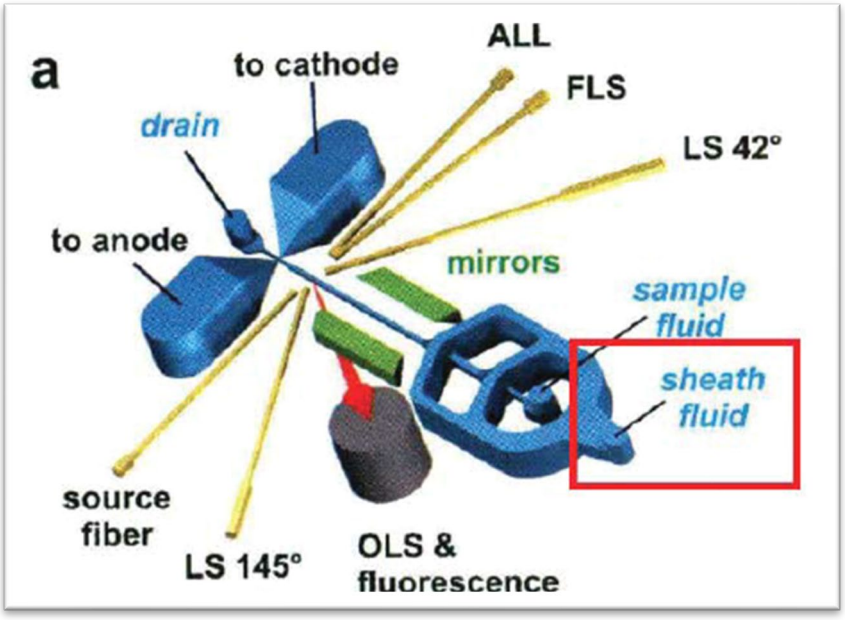
Finally, Simonnet in combination with Kummrow render claim 9 obvious. Aside from its erroneous determination as to claim 1, the Board properly gave no indication that claim 9 is not obvious. (*See* Appx58–59.)

Claim 9 discloses the "microfluidic assembly of claim 1," with the additional requirement that "each of the fluid focusing features is in fluid communication with a first focusing fluid inlet port provided on a top surface of the substrate." (Appx96(19:40–43).) The patent does not define the term "in fluid communication with." The term's plain meaning, however, indicates that claim 9 requires that the fluid focusing features be connected by a fluid path to a common "inlet" for receiving focusing fluid. (Appx2088–2089.) This path would allow

focusing fluid to enter the device through a single inlet and then be disbursed to the different fluid focusing features.

Simonnet does not disclose this feature. Instead, the High-Throughput Device provides a separate inlet for each of the fluid focusing features. (Appx2089–2090; Appx2118–2119.) But it is well known in the field of microfluidics that a requirement like claim 9's can be accomplished by "serially arrang[ing] fluid focusing features" so that they can be connected by a single pathway to an inlet for focusing fluid. (Appx2090; *see* Appx2001–2004.)

Kummrow does exactly this, using a single inlet for focusing fluid, which then distributes the focusing fluid to the three focusing features through interconnected sheath flow channels. (*See* Appx2139; Appx2090–2093.)



(Appx2091 (annotating Appx2139(fig. 1(a))).)

This design allows for "easy handling" by the device operator, who does not need to inject focusing fluid at multiple points. (Appx2139; *see* Appx2819 (noting that having to make "sensitive adjustment[s]" of multiple focusing fluids, as required by the Simonnet design, "can cause some inconvenience in daily use"). Kummrow expressly discusses Simonnet as one of the "most advanced structures" for multidimensional focusing, while noting that Simonnet's design uses multiple inlets for focusing fluid. (Appx2139 & n.22.) Kummrow sought to improve upon the Simonnet design and others like it, including by providing for a common inlet for focusing fluid.

Combining Simonnet and Kummrow to achieve the requirement of claim 9 would have been obvious to a skilled artisan. (*See* Appx2090–2093.) Reducing the number of inlets for focusing fluid, as Kummrow did, was one of a "finite number of predictable options" that a skilled artisan would have tried through ordinary creativity. *See C.R. Bard, Inc.*, 2021 WL 3574043, at *7; *Sealy Tech., LLC v. SSB Mfg. Co.*, 825 F. App'x 801, 807 (Fed. Cir. 2020) (concluding that the number of handles in a mattress design was "merely a trivial difference," and thus "a designer of ordinary skill would have been motivated to incorporate" the prior art's design (citation omitted)). As a result, claim 9 is unpatentable.

CONCLUSION

The Court should, at the very least, vacate the Board's decision because the Board relied on an erroneous construction of the phrase "the sample stream" in claim 1. But because all challenged claims are unpatentable on any construction of that term, the Court should reverse.

August 15, 2022

Respectfully submitted,

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ADDENDUM

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ABS GLOBAL, INC.,
GENUS, PLC,
Petitioner,

v.

CYTONOME/ST, LLC,
Patent Owner.

IPR2021-00088
Patent 10,583,439 B2

Before LYNNE H. BROWNE, SCOTT A. DANIELS, and JAMES A. WORTH, *Administrative Patent Judges.*

WORTH, *Administrative Patent Judge*.

DECISION
Final Written Decision
Determining No Challenged Claims Unpatentable
35 U.S.C. § 318(a)

I. INTRODUCTION

This is a Final Written Decision in an *inter partes* review that addresses claims 1, 2, 6, 8, and 9 (the "challenged claims") of U.S. Patent No. 10,583,439 B2 (Ex. 1001, "the '439 patent"). We have jurisdiction under 35 U.S.C. § 6(b). This Final Written Decision is issued pursuant to 35

U.S.C. § 318(a). Having reviewed the arguments of the parties and the supporting evidence, we find that Petitioner has not demonstrated by a preponderance of the evidence that claims 1, 2, 6, 8, and 9 are unpatentable.

A. Background and Summary

On October 26, 2020, ABS Global, Inc. and Genus, PLC (collectively, "Petitioner") filed a Petition (Paper 1, "Pet.") requesting an *inter partes* review of claims 1, 2, 6, 8, and 9 of the '439 patent. On February 10, 2021, Cytonome/ST, LLC ("Patent Owner") filed a Preliminary Response (Paper 8, "Prelim. Resp."). With authorization, the parties filed further pre-institution briefing related to the construction of the term "fluid focusing region" and collateral estoppel, as follows. On March 5, 2021, Petitioner filed a reply to the Preliminary Response (Paper 12, "Prelim. Reply"). On March 12, 2021, Patent Owner filed a sur-reply (Paper 14, "Prelim. Sur-Reply").

On May 4, 2021, we instituted an *inter partes* review of claims 1, 2, 6, 8, and 9 of the '439 patent. Paper 18 ("Dec. Inst.").

On July 29, 2021, Patent Owner filed a Response to the Petition. Paper 21 ("PO Resp."). On October 21, 2021, Petitioner filed a Reply. Paper 23. On December 10, 2021, Patent Owner filed a Sur-reply. Paper 27.

On February 2, 2022, an oral argument was held in the proceeding, a transcript of which has been entered in the record. Paper 37 ("Tr.").

On February 4, 2022, we issued an Order (Paper 34) inviting supplemental briefing on claim construction for claim 1, e.g., on whether claim 1 requires that the device of claim 1 be in any particular orientation to meet the claim.

On February 11, 2022, Petitioner filed a supplemental brief on claim construction. Paper 35 ("Pet. Supp. Br."). On February 18, 2022, Patent Owner filed a supplemental brief on claim construction. Paper 38 ("PO Supp. Br.").

Petitioner relies on the declaration and supplemental declaration of David Issadore, Ph.D. Ex. 1003; Ex. 1054. Patent Owner relies on the declaration and supplemental declaration of Don W. Arnold, Ph.D. Ex. 2001; Ex. 2006.

B. Real Parties-in-Interest

Petitioner identifies ABS Global, Inc. and Genus PLC as real parties-in-interest. *See* Pet. 3. Patent Owner identifies Cytonome/ST, LLC as the real-party-in-interest. Paper 5, 1.

C. Related Matters

The parties note as related litigation in federal district court, *Inguran, LLC v. ABS Global, Inc.*, No. 3:20-cv-00085-wmc (W.D. Wis., filed Mar. 18, 2020) and *Inguran, LLC v. ABS Global, Inc.*, No. 3:20-cv-00349-wmc (W.D. Wis., filed Apr. 15, 2020). *See* Pet. 3–4; Paper 5, 1.

D. The '439 Patent

The '439 patent is titled "Hydrodynamic Focusing Apparatus and Methods" and relates to "hydrodynamic focusing, in particular, in a microfluidic device," and more specifically relates to "systems and methods for producing a sheath flow in a flow channel and, in particular, in a micro channel in a microfluidic device." Ex. 1001, code (54), 1:14–19.

According to the '439 patent, sheath flow is a particular type of laminar flow in which one layer of sample fluid, or a particle, is surrounded by another layer of focusing fluid on more than one side. *Id.* at 1:23–25. The '439 patent discloses that the process of confining a particle stream in a

fluid is referred to as a "sheath flow" configuration. *Id.* at 1:25–27. The '439 patent describes an example with a single file row:

> For example, in a sheath flow configuration, a sheath fluid may envelop and pinch a sample fluid containing a number of particles. The flow of the sample fluid containing particles suspended therein may be narrowed almost to the outer diameter of particles in the center of the sheath fluid. The resulting sheath flow flows in a laminar state within an orifice or channel so that the particles are aligned and accurately pass through an orifice or channel in a single file row.

Id. at 1:29–35.

In its Background section, the '439 patent states that then-conventional devices that had been employed to implement sheath flow had relatively complex designs and were relatively difficult to fabricate. *Id.* at 1:54–56.

Figure 3A of the '439 patent, reproduced below, depicts an embodiment of such a device:

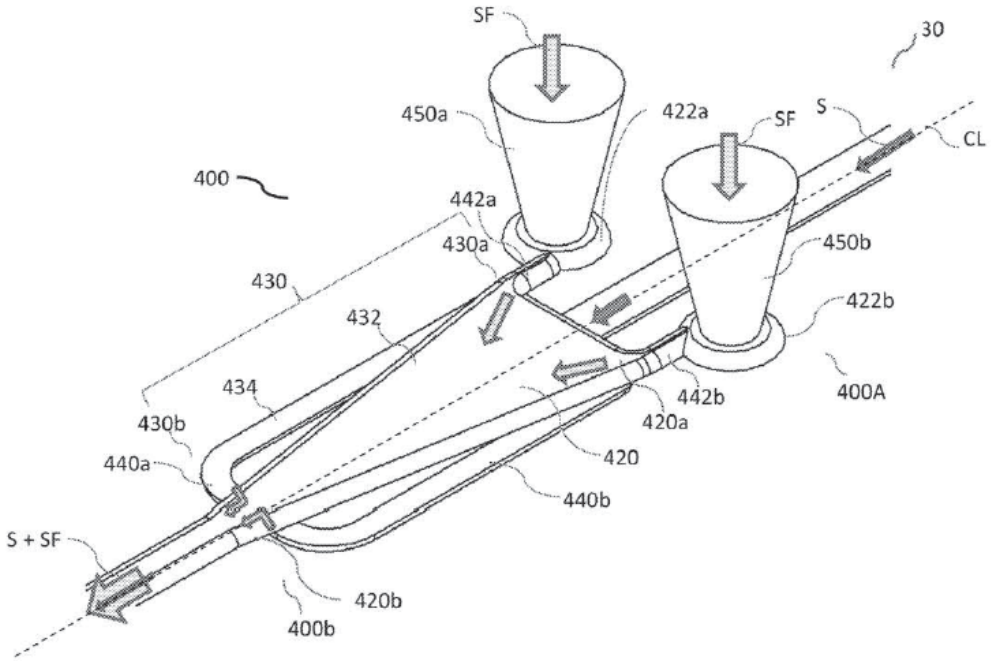


Figure 3A is a top perspective view of a portion of a flow channel geometry with arrows schematically depicting flow of sample fluid S along centerline CL and focusing fluid SF. *Id.* at 4:7–10.

Figure 3A shows a portion of micro channel 30 having core stream forming geometry 400. *Id.* at 10:11–12. Core stream forming geometry 400 may be fabricated in plastics, polycarbonate, glass, metals, or other suitable materials using microfabrication, injection molding, stamping, machining, 3D printing or by other suitable fabrication techniques. *Id.* at 10:38–41. As such, core stream forming geometry 400 may be formed in a single substrate layer, or by a plurality of stacked layers. *Id.* at 10:41–43.

Sample fluid S flowing through micro channel 30 may enter core stream forming geometry 400 along longitudinal centerline CL. *Id.* at 10:13–17. Focusing fluid may enter the core stream forming geometry 400 at upstream region 400a of the core stream forming geometry and also at downstream region of the core stream forming geometry 400b. *Id.* at 10:20–24.

Two sheath inlet ports 450a, 450b are associated with micro channel 30. *Id.* at 10:54–55. Sheath fluid channels 440a and 440b extend, respectively, from sheath fluid inlet ports 450a and 450b. *Id.* at 10:58–60. Each sheath fluid channel 440 extends from upstream region 430a of the fluid focusing region 430 to downstream region 430b of the fluid focusing region 430. *Id.* at 10:60–62. Each sheath fluid channel 440 is configured to transport focusing fluid SF from sheath inlet port 450 to micro channel 20. *Id.* at 10:63–66.

Lateral fluid focusing chamber 420 is widest at its upstream end 420a and narrowest at its downstream end 420b. *Id.* at 11:24–26. Between upstream end 420a and downstream end 420b, the chamber 420 substantially

linearly tapers symmetrically with respect to the centerline CL in the lateral direction. Between upstream end 420a and downstream end 420b, the chamber 420 has a substantially constant thickness. *Id.* at 11:26–32.

Sample fluid S enters focusing chamber 420 from below via a symmetrically centered opening having a length equal to overlap OL region and a width equal to the width of micro channel 30. *Id.* at 11:60–63. As the chamber 420 narrows or tapers in the lateral direction as the fluid travels downstream, an increasing inward force from the lateral sides of the chamber 420 acts on the fluid within the chamber, thus tending to focus (e.g., constrict) sample S in the middle of the lateral fluid focusing chamber 420. *Id.* at 12:3–8. The increasing inward force further tends to accelerate both the sheath and the sample within fluid focusing region 430 in flow channel 30. *Id.* at 12:8–11.

At downstream end 420b of lateral fluid focusing chamber 420, the vertical fluid focusing component provides a vertical upwardly-directed focusing force. *Id.* at 12:12–14. Specifically, vertical fluid focusing channels 440a, 440b introduce focusing fluid SF[1] from inlet ports 450a, 450b into lateral fluid focusing chamber 420 at downstream end 420b. *Id.* at 12:14–17. Thus, the vertical fluid focusing channels 440a, 440b introduce focusing fluid SF into fluid focusing chamber 420 at vertical focusing flow inlet 446 from below. *Id.* at 12:23–26.

[1] The Specification uses the term "FS" here but we understand this to be a typographical error and to refer to focusing fluid SF, as is used elsewhere. *See id.*

Figure 3D of the '439 patent, reproduced below, depicts a cross-sectional view of the same embodiment as Figure 3A (*see id.* at 4:19–21):

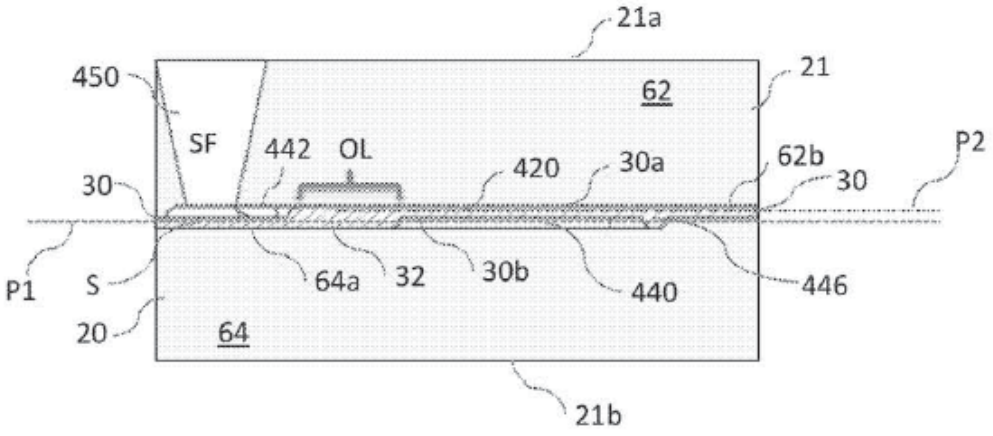


Figure 3D is a cross-sectional view through a line marked in Figure 3C (which is not reproduced here), i.e., a cross-sectional view through what appears to be a line down the middle of the microfluidic unit. *See id.* at 4:19–21, Fig. 3C.

As depicted in Figure 3D, sample S enters the fluid focusing region 430 at upstream end 430a in plane P1 below plane P2 in which the lateral fluid focusing chamber 420 is located. *Id.* at 12:37–40. Sample S is directed upward from plane P1 into plane P2 of lateral focusing chamber 420 in the overlapped region OL. *Id.* at 12:40–43. Then, at downstream end 430b of fluid focusing region 430, the laterally focused sample within a sheath of focusing fluid (S+SF) is vertically focused upward by the introduction of focusing fluid SF at vertical focusing flow inlet 446 from below. *Id.* at 12:43–47.

Figure 6A of the '439 patent is reproduced below, with coloring and annotations added (*see* Pet. 15):

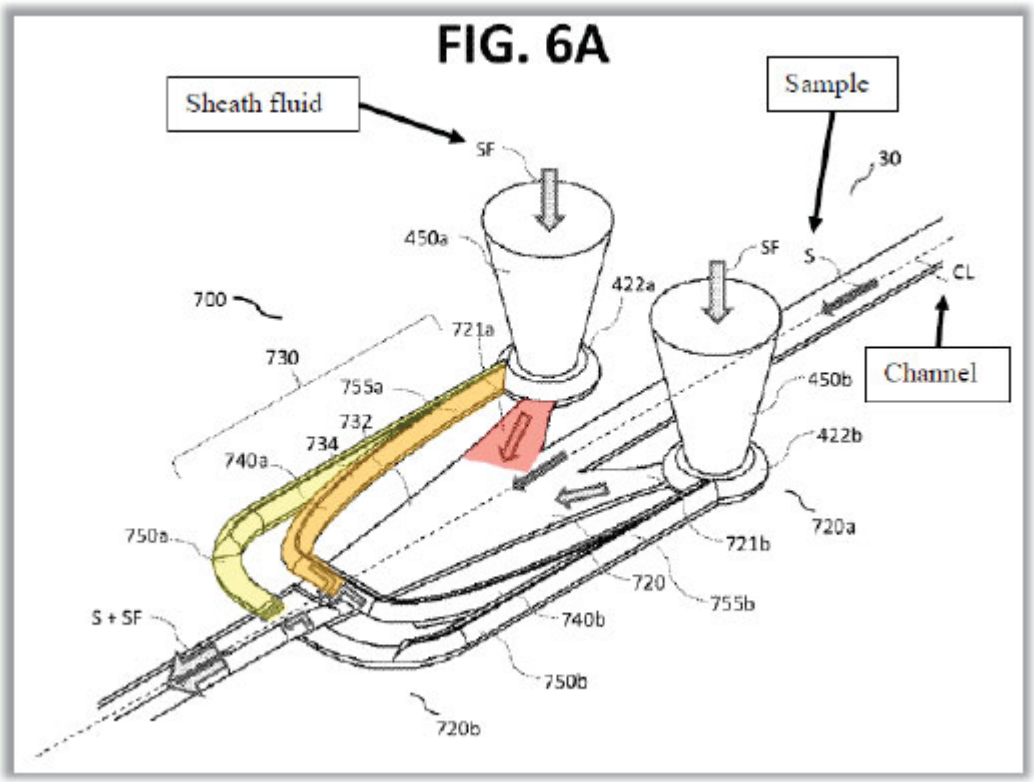


Figure 6A is a top perspective view of a portion of a flow channel geometry with arrows schematically depicting flow of sample fluid and focusing fluid in accordance with certain embodiments. *Id.* at 4:46–49. This figure has, among other features, lateral fluid focusing chamber 720 with tapered inlet 721 (shown with red highlighting), as well as first and second sets of vertical fluid focusing channels 740, 750 (shown with orange and yellow highlighting). *See id.* at 17:1–8.

E. Illustrative Claim

Claim 1, reproduced below, is the sole independent claim challenged in the Petition and is illustrative of the subject matter:

> 1. A microfluidic assembly for use with a particle processing instrument, the microfluidic assembly comprising:

a substrate; and

a flow channel formed in the substrate, the flow channel having:

an inlet configured to receive a sample stream;

a fluid focusing region ***configured to focus the sample stream***, the fluid focusing region having a lateral fluid focusing feature, a first vertical fluid focusing feature, and a second vertical fluid focusing feature, the lateral, the first vertical, and the second vertical fluid focusing features provided at different longitudinal locations along the flow channel, wherein a bottom surface of the flow channel lies in a first plane upstream of the first and second vertical fluid focusing features and the bottom surface of the flow channel shifts vertically upward to lie in a second plane downstream of the first and second vertical focusing features; and

an inspection region at least partially downstream of the fluid focusing region.

Ex. 1001, 18:43–63 (emphasis added).

F. Prior Art and Asserted Grounds

Petitioner asserts that claims 1, 2, 6, 8, and 9 would have been unpatentable on the following grounds (Pet. 4):

Claim(s) Challenged	35 U.S.C. §	Reference(s)/Basis
1, 2, 6, 8	102[2]	Simonnet[3]

[2] The Leahy-Smith America Invents Act ("AIA"), Pub. L. No. 112-29, 125 Stat. 284, 287–88 (2011), amended 35 U.S.C. §§ 102 and 103. It is undisputed on this record that the priority date of the '439 patent is March 14, 2013. *See* Pet. 5–6. Because the application leading to the '439 patent was filed before the effective date of the relevant amendment, the pre-AIA version of §§ 102 and 103 applies.

[3] Claire Simonnet and Alex Groisman, *High-Throughput and High-Resolution Flow Cytometry in Molded Microfluidic Devices*, 78 Anal. Chem. 5653–5663 (2006) (Ex. 1005, "Simonnet").

Claim(s) Challenged	35 U.S.C. §	Reference(s)/Basis
1, 2, 6, 8	103	Simonnet
8	103	Simonnet, Kim[4]
9	103	Simonnet, Kummrow[5]

II. ANALYSIS

A. Legal Standards

A "prior art reference—in order to anticipate under 35 U.S.C. § 102—must not only disclose all elements of the claim within the four corners of the document, but must also disclose those elements 'arranged as in the claim.'" *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1369 (Fed. Cir. 2008) (quoting *Connell v. Sears, Roebuck & Co.*, 722 F.2d 1542, 1548 (Fed. Cir. 1983)). "A single prior art reference may anticipate without disclosing a feature of the claimed invention if such feature is necessarily present, or inherent, in that reference." *Allergan, Inc. v. Apotex Inc.*, 754 F.3d 952, 958 (Fed. Cir. 2014).

A patent claim is unpatentable under 35 U.S.C. § 103 if the differences between the claimed subject matter and the prior art are such that the subject matter, as a whole, would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. 35 U.S.C. § 103; *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007). "[W]hen a patent claims a structure already known in the prior art that is altered by the mere substitution of one element for

[4] Dong Sung Kim et al., *An efficient 3-dimensional hydrodynamic focusing microfluidic device by means of locally increased aspect ratio*, 86 Microelectronic Engineering 1343–1346 (2009) (Ex. 1015, "Kim").
[5] A. Kummrow et al., *Microfluidic structures for flow cytometric analysis of hydrodynamically focussed blood cells fabricated by ultraprecision micromachining*, 9 Lab Chip 972–981 (2009) (Ex. 1006, "Kummrow").

another known in the field, the combination must do more than yield a predictable result." *KSR*, 550 U.S. at 416 (citing *United States v. Adams*, 383 U.S. 39, 50–51 (1966)). The question of obviousness is resolved based on underlying factual determinations including: (1) the scope and content of the prior art; (2) any differences between the claimed subject matter and the prior art; (3) the level of ordinary skill in the art; and (4) objective evidence of non-obviousness.[6] *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

B. Level of Ordinary Skill in the Art

Petitioner argues that a person of ordinary skill in the art (POSA) at the relevant time would have had a bachelor's or master's degree in the field of bioengineering, mechanical engineering, chemical engineering, or analytical chemistry, or a related field and at least three years of experience in designing or developing microfluidic systems. Pet. 4. Petitioner also argues, *inter alia*, that a skilled artisan would have been familiar with the asserted art and would have known how to configure those systems to focus particles in a variety of directions. *See id.* at 5 (citing Ex. 1003 ¶ 75).

In the Decision on Institution, we set forth our preliminary determination that a person of ordinary skill in the art at the relevant time would have had a bachelor's or master's degree in the field of bioengineering, mechanical engineering, chemical engineering, or analytical chemistry, or a related field and at least three years of experience in designing or developing microfluidic systems. *See* Dec. 10–11. Patent Owner does not dispute this definition. *See* PO Resp. 4.

[6] The parties do not present or address objective evidence of non-obviousness in this proceeding.

After considering all evidence and arguments anew, we determine that it is proper to maintain our definition of the level of skill in the art.

C. Claim Construction

We construe each claim "in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent." 37 C.F.R. § 42.100(b). Under this standard, claim terms are generally given their plain and ordinary meaning as would have been understood by a person of ordinary skill in the art at the time of the invention and in the context of the entire patent disclosure. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed. Cir. 2005) (*en banc*).

Petitioner requests construction of the terms "focusing" and "fluid focusing feature." Pet. 17–22. In the Decision on Institution, we provided preliminary construction of the terms "focusing" and "fluid focusing region." Dec. Inst. 11–21. Patent Owner requests that the Board revise its construction of "focusing" and also makes certain arguments about focusing in the claim phrase "configured to focus the sample stream"; requests that the Board revise its construction of "focusing region"; and argues that it is not necessary to construe "fluid focusing feature" but provides arguments in the event that the Board reaches that issue. PO Resp. 4–17. We address the construction of "focusing," "configured to focus the sample stream," and "fluid focusing region" below. *See Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999) (claims are construed only to the extent necessary to resolve a dispute).

We also solicited post-hearing briefing on the following questions, which we also address, in part, to the extent necessary to resolve this dispute:

> 1. Does the language from the Specification of the '439 patent (Ex. 1001, 6:4–10) mean that a device can meet the claim if oriented in any manner, as a matter of claim construction?
>
> 2. If not, and a device can only meet the claim in one orientation, does that create a problem under *IPXL*, i.e., where there would be a mixing of a device and method of use in a claim? *See IPXL Holdings, L.L.C. v. Amazon.com, Inc.*, 430 F.3d 1377, 1384 (Fed. Cir. 2005).
>
> 3. If a device can only meet the claim in one orientation, does that create uncertainty as a matter of notice to the public about whether a device meets the claim, e.g., if the same device has been made, used, or sold in a different orientation?
>
> 4. Is the "wherein" clause part of the "configured to" clause? If so, does that support an understanding that the device meets the claim in any orientation?

Paper 34, 3.

1. "focusing"; "configured to focus the sample stream"

Petitioner argues that "focusing" should be construed to mean "narrowing, pinching, or otherwise confining a sample fluid with sheath fluid." Pet. 17 (citing Ex. 1003 ¶¶ 98–101). Petitioner asserts that focusing was a term commonly used in the art with this meaning. *Id.* at 17–18 (citing Ex. 1003 ¶ 101; Ex. 1031, 167; Ex. 1013[7], 247; Ex. 1007[8], 3863; Ex. 1011[9],

[7] Albert Folch, *Introduction to BioMEMS*, CRC Press (2013) (Ex. 1013, "Folch"). It is unclear on this record whether Folch is prior art or merely a reference that is contemporaneous with the '439 patent. In either event, we determine that Folch may be used as evidence of the meaning of the term "focus" to a person of ordinary skill at the time of the invention.

[8] Knight et al., *Hydrodynamic Focusing on a Silicon Chip: Mixing Nanoliters in Microseconds*, 80(17) Physical Review Letters 3863 (Apr. 27, 1998) (Ex. 1007, "Knight").

[9] Wada et al., U.S. Patent No. 6,506,609 B1, iss. Jan. 14, 2003 (Ex. 1011).

8:11–14). Petitioner argues that the '439 patent uses the term "focusing" in a manner consistent with its ordinary meaning and that the applicants gave no indication that they intended to depart from the ordinary meaning. *Id.* at 18 (citing Ex. 1001, 1:27–29, 10:29–31, 12:5–7, 12:12–14, 15:42–46; Ex. 1003 ¶¶ 98–101).

In the Decision on Institution, we set forth our preliminary construction of "focusing" to mean "pinching, confining, squeezing, or constricting." Dec. 14. In particular, we reviewed contemporaneous and prior art usages where "focusing" was variously referred to as "pinching" or "squeezing" and found this to be consistent with the disclosure in the Specification that an increasing force from the lateral sides is "*tending to focus (e.g., constrict)*" the sample. *See* Dec. Inst. 14 (citing Ex. 1001, 12:3–8; Ex. 1013, 247; Ex. 1011, 8:11–14) (emphasis in Decision).

Patent Owner argues that "focusing" requires a "sheath flow" with only a single sample stream, and requests that the Board revise its construction to "pinching, confining, squeezing, or constricting a single sample stream with sheath flow." PO Resp. 5–11.

a) Whether "focusing" requires "sheath flow"

Patent Owner argues that the Specification describes using "sheath flow" to focus a sample stream in every embodiment (citing Ex. 1001, 12:62–13:2, 13:36–38; Ex. 2006 ¶¶ 39–47); that the background section of the Specification contains a definition of sheath flow (citing Ex. 2006 ¶¶ 10, 35 (quoting Ex. 1001, 1:23–25); that Dr. Issadore conceded during cross-examination that he could not see or imagine focusing without sheath flow (citing Ex. 2008, 92:11–20, 187:14–188:9, 189:14–24; 190:16–191:3); that an authority relied on by Petitioner's expert uses the terms hydrodynamic focusing and sheath flow interchangeably (citing Ex. 2006 ¶¶ 11, 38, 48–52;

Ex. 1032, 148); and that a person of ordinary skill sought to achieve sheath flow so that the particles in a sample can be accurately inspected in a flow cytometer (citing Ex. 2006 ¶¶ 12–14, 38). PO Resp. 4–7.

Petitioner argues that "sheath flow" is used in different ways in the art, where sometimes it is used interchangeably with "sheath fluid" and other times it is used to refer to a laminar-flow configuration where one layer of fluid surrounds another on more than one side. Pet. Reply 2, 4–5 (citing Ex. 1054 ¶¶ 4, 6–8; Ex. 1038 ¶ 135; Ex. 1053, 88:4–15, 95:7–14; Ex. 1055, 4555; Ex. 1003 ¶ 47).[10] Petitioner argues that Dr. Issadore testified that "focusing" requires "sheath fluid," and "insomuch as sheath flow is sheath fluid . . . flowing in a laminar way," then you "couldn't have focusing without—without that" (citing Ex. 2008, 92:11–20, 92:24–95:10). Pet. Reply 3. Petitioner argues that Dr. Issadore did not testify that "focusing" requires surrounding a sample on multiple sides with sheath, or that it forecloses a split sample. *Id.*

Petitioner argues that skilled artisans routinely used the term "focusing" to describe introducing sheath fluid to confine a sample from just one side, which would not meet the sheath flow requirement advanced by Patent Owner. Pet. Reply 4–5 (citing, e.g., Ex. 1054 ¶¶ 8–9; Ex. 1039, Fig. 3; Ex. 1040, 26–27, Fig. 1, 2; Ex. 1041, 3746, Figs. 2, 3; Ex. 1042, 6:54–57, Fig. 4). Petitioner argues that Wada is an example of the prior art where there was "focusing" but only sheath fluid on one side and thus not sheath flow. Pet. Reply 4 (citing, e.g., Ex. 1011, 9:40–45, Figs. 16, 23, 24).

[10] Petitioner argues that Patent Owner argues for a third definition of sheath flow, i.e., one and only one layer of sample fluid surrounded by sheath fluid. Pet. Reply 2–3. We address below whether focusing requires a single stream.

Before we address whether "focusing" requires sheath flow, we first address what is meant by sheath flow. We use the definition of "sheath flow" found in the Specification, i.e., sheath flow is a particular type of laminar flow in which one layer of sample fluid, or a particle, is surrounded by another layer of focusing fluid on more than one side. Ex. 1001, 1:23–25.

Nevertheless, just because the Specification contains a definition of sheath flow does not mean that "focusing" requires sheath flow. In other words, the Specification does not contain a definition of "focusing" that defines "focusing" as requiring "sheath flow." As discussed above, the Specification does state "focusing (e.g., constricting)." *See also* Tr. 41:22–42:8 (discussing the Specification of the '439 patent). Whether or not this disclosure rises to the level of a definition, it indicates that focusing is understood to be constricting and does not mention "sheath flow." Although sheath flow may, in certain cases, be a natural result of having multiple fluid focusing features (*see* Ex. 1001, 14:6), the claim itself does not require that focusing include sheath flow.[11]

[11] In terms of resolving the dispute between the parties, the real question appears to be whether the sample stream that exits the focusing region must be a single sample stream or whether it may encompass split sample streams, a question that we address in the next subsection. Patent Owner's ultimate argument appears to be that "focusing" requires a single sample stream because "focusing" requires sheath flow and sheath flow requires a single sample stream. However, we agree with Petitioner that persons of ordinary skill could still refer to split streams as having "sheath flow" (*see* Ex. 1054 ¶ 11; Ex. 1043, 2215, Fig. 10 and caption ("separated two-sample 3D sheath flow"); Ex. 1044, 2 ("multiple core-sheath flow"), and therefore, even if we agreed with Patent Owner that focusing requires sheath flow, that would not clarify whether "focusing" precludes split sample streams. In any event, we conclude that focusing does not require sheath flow.

We recognize that there are examples in the Specification where there is sheath flow. *E.g.*, Ex. 1001, 5:30–32 ("Certain embodiments described herein relate [to] systems and methods for producing a sheath flow . . ."), 10:35–37 ("The illustrated core stream forming geometry 400 provides improved sheath flow capabilities and thus improved sample focusing capabilities."). However, case law generally counsels against importing limitations from the Specification into the claims. *See, e.g., Phillips v. AWH Corp.*, 415 F.3d 1303, 1323 ("To avoid importing limitations from the specification into the claims, it is important to keep in mind that the purposes of the specification are to teach and enable those of skill in the art to make and use the invention and to provide a best mode for doing so.").[12] We, therefore, do not import "sheath flow" into the definition of focusing.

Further, as Petitioner observes (Pet. Reply 5 n.3), the applicant in the prosecution history considered but ultimately did not use claim language that would have expressly limited a claim to "sheath flow." *See* Ex. 1002, 1074 (claim 12). The original claim proposed during prosecution read "The microfluidic assembly of claim 1, wherein the flow channel further

[12] We recognize that there are certain instances in the case law where the Specification could give rise to an implied disclaimer, e.g., where the Specification disparages the presence of a feature in the prior art and where there is the absence of the feature in every embodiment. *See In re Abbott Diabetes Care Inc.*, 696 F.3d 1142, 1149 (Fed. Cir. 2012). However, it is preferred that the Specification expressly define a special usage of a claim term and expressly disclaim features. Here, the Specification states "*Certain* embodiments described herein relate [to] systems and methods for producing sheath flow" (Ex. 1001, 5:30–32) (emphasis added), and although this discussion appears to relate to the claimed embodiment, we note that the language "[c]ertain embodiments" is not the language of a disclaimer that would apply to all cases.

comprises a sheath inlet in fluid communication with the sheath source, and wherein a sample inlet is positioned within a sheath flow created by the sheath inlet to facilitate a co-axial flow of sheath and sample." *Id.* Thus, the applicant could have chosen to expressly claim "sheath flow" and did not do so.

Our conclusion is consistent with the usage in the art, such as Wada, where focusing is described to occur even with sheath fluid being applied on one side of a sample stream to pinch the sample stream. *See* Ex. 1054 ¶ 7 (discussing Ex. 1011, 7:15–17, 9:40–45, Fig. 16); Ex. 1011, 9:40–52. Figure 16 of Wada, annotated by Dr. Issadore (Ex. 1054 ¶ 7), is reproduced below:

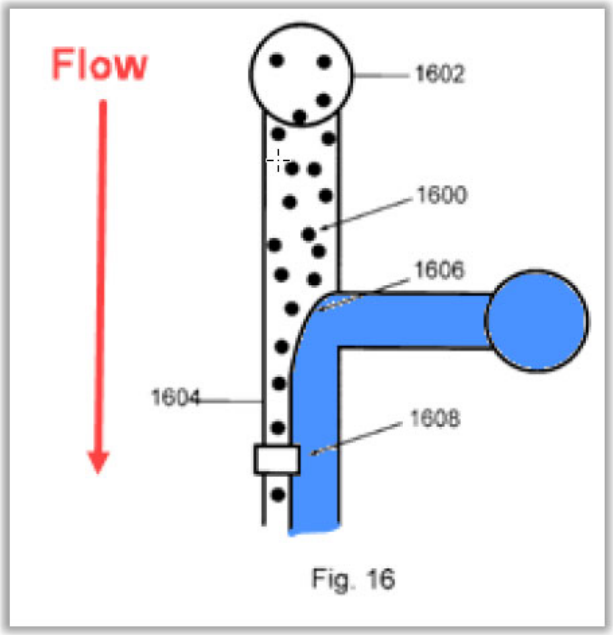


Figure 16 of Wada shows a T-junction microchannel configuration for hydrodynamically focusing cells for use in, e.g., flow cytometry. Ex. 1011, 7:15–17. Dr. Issadore's annotation shows an arrow designating the direction of flow in red, and stream 1606 in blue that is used to focus particles 1600. *See* Ex. 1011, 9:40–52.

Patent Owner relies on its cross examination of Dr. Issadore to contend that "focusing" requires "sheath flow." However, based on our review of the deposition testimony, Dr. Issadore's concession is not as clear as Patent Owner argues. The discussion of "sheath flow" occupies several pages, but the central passage appears to be as follows:

> [COUNSEL] . . . Using your understanding of "sheath flow" and "focusing," can a microfluidic device focus a sample without using sheath flow?
>
> [DR. ISSADORE] Under the definition I give in Paragraph 98, you couldn't have focusing without sheath fluid. And insomuch as sheath flow is sheath fluid in a -- you know, flowing in a laminar way, as described in the previous paragraph we discussed, then I -- then you couldn't. You couldn't have focusing without -- without that.

Ex. 2008, 92:11–20. Dr. Issadore's reference to paragraph 98 appears to refer to paragraph 98 of his initial declaration, which is the subject of the deposition. *See* 37 C.F.R. § 42.51(b)(1)(ii). Paragraph 98 of the declaration contains a definition of "focusing" as follows: "The ordinary meaning in the context of the '439 Patent of the term '*focusing*' is 'narrowing, pinching, or otherwise confining a sample fluid with sheath fluid.'" Ex. 1003 ¶ 98. It is true that earlier in the deposition, Dr. Issadore agreed to apply a definition of sheath flow for the deposition: "'Sheath flow is a particular type of laminar flow in which one layer (for example, the sheath) surrounds another layer (for example, a sample) on more than one side.'" *See* Ex. 2008, 90:23–91:9. However, in the portion of the deposition reproduced and indented above, Dr. Issadore appears to use a different definition of "sheath flow," i.e., "insomuch as sheath flow is sheath fluid in a -- you know, flowing in a laminar way." *See* Ex. 2008, 92:11–20. Further, the definition that Dr. Issadore refers back to, i.e., "in the previous paragraph we discussed,"

appears to be the definition of "focusing" in paragraph 98 of the declaration and not the definition of "sheath flow" discussed earlier in the deposition. Accordingly, all that Dr. Issadore clearly concedes to is that focusing involves sheath fluid flowing in a laminar manner. We agree with Petitioner that Dr. Issadore did not indicate agreement with Patent Owner that "focusing" requires sheath flow in the sense that sheath fluid must surround a layer of sample on more than one side.

We also agree with Petitioner that the Specification uses "SF" to refer to both sheath flow and sheath fluid, and thus uses the terms interchangeably in places. For example, the Specification refers to fluid SF sometimes as "focusing fluid SF" and sometimes as "sheath flow SF." *See, e.g.,* Ex. 1001, 10:17 ("focusing fluid SF"), 12:65 ("sheath flow SF"), 13:11 ("sheath flow SF"), 13:16 ("sheath flow SF"). Therefore, the Specification is of limited assistance in determining whether it is only sheath fluid or also sheath flow that is required.

Thus, we determine that there is insufficient evidence to import a requirement of sheath flow into the definition of focusing.

b) Whether "focusing" requires a single stream

Patent Owner also argues that focusing requires focusing into a single stream based on its arguments that every embodiment in the Specification produces a focused core stream along the centerline of the channel (citing Ex. 1001, 5:27–29, 10:12–16, 10:28–31, 12:65–13:2, 13:48–58, 16:62–65, Figs. 4A–6D; Ex. 2006 ¶¶ 19, 44, 54, 56–60, 63); that the presence of a second stream would block the sorting of the selected particle in a subsequent particle sorting region (citing Ex. 1001, 5:15–29, 18:6–11; 13:53–58; Ex. 2001 ¶¶ 67, 86–89, 141; Ex. 2006 ¶¶ 14, 19, 20, 36, 65); and that in the presence of a second stream, particle detection accuracy decreases

as the sheath fluid flow rate increases (citing Ex. 2006 ¶ 17). PO Resp. 8–10. Patent Owner also argues that the language of claim 2 referring to "a centerline of the sample stream" indicates that the sample stream of claim 1, from which claim 2 depends, is a single sample stream, and that it would be absurd to have a centerline of two sample streams. PO Resp. 11 (citing *Wright Med. Tech., Inc. v. Osteonics Corp.*, 122 F.3d 1440, 1445 (Fed. Cir. 1997); *AIA Eng'g Ltd. v. Magotteaux Int'l S/A*, 657 F.3d 1264, 1276 (Fed. Cir. 2011) ("We strive, where possible, to avoid nonsensical results in construing claim language."); Ex. 2006 ¶¶ 61–64). Patent Owner also argues that the term "the sample stream" in claim 1 is singular. PO Sur-reply 2.

Petitioner argues that skilled artisans have long recognized that a device can "focus" a sample even if it splits the sample into multiple sub-streams, and that this may even be desirable for certain applications. PO Resp. 5–6 (citing Ex. 1054 ¶ 10; Ex. 1014, 1483; Ex. 1020, 114104-2; Ex. 1005, 5657; Ex. 1039, 326). Petitioner argues that "sheath flow" is not part of a proper construction of "focusing," but even if "sheath flow" were required, skilled artisans routinely used "sheath flow" to describe devices with split- or multiple-core samples surrounded by sheath fluid. Pet. Reply 6 (citing Ex. 1054 ¶ 11; Ex. 1043, Fig. 10, 2215; Ex. 1044, 2; Ex. 1045, 14:55–57).

Petitioner argues that dependent claim 2 provides no justification for departing from the ordinary meaning of "focusing" because, according to Petitioner, even a split sample has a centerline, which can be "at the center of flow channel," i.e., between the two branches of the split sample. Pet.

Reply 7 (citing Pet. 58; Ex. 1003 ¶ 194; Ex. 1054 ¶ 12; Ex. 1005, 5657; Ex. 1020, 114104-2; Ex. 1014, 1483)).[13]

[13] Patent Owner also makes certain arguments that a split stream would be disfavored if the purpose of the device were to detect sample or to accelerate the rate of flow. *See, e.g.,* PO Resp. 1 ("accuracy in the system decreases sharply as the sheath flow rate increases which is the opposite of what happens with focusing"), 7 (citing, e.g., Ex. 2006 ¶¶ 12–14, 38). Petitioner disagrees, and points to testimony of Dr. Issadore that a measure of accuracy increased as the relative sheath flow rate increased. Pet. Reply 18–19 (Ex. 1054 ¶ 27); Ex. 2006 ¶ 80. We agree that f_d/f_0 [the ratio of the number of particles detected per second to the total particle fluxes] is greatest for the highest relative sheath flow rate [the flow rate of the sheath fluid relative to the flow rate of the sample] as described in Dr. Issadore's Reply Declaration. *See* Ex. 1054 ¶¶ 23–24, 26–27 (citing, e.g., Ex. 1005, 5659). In any event, we determine that Patent Owner's arguments regarding accuracy go to the purpose of the claimed invention but do not, on their own, limit the claims.

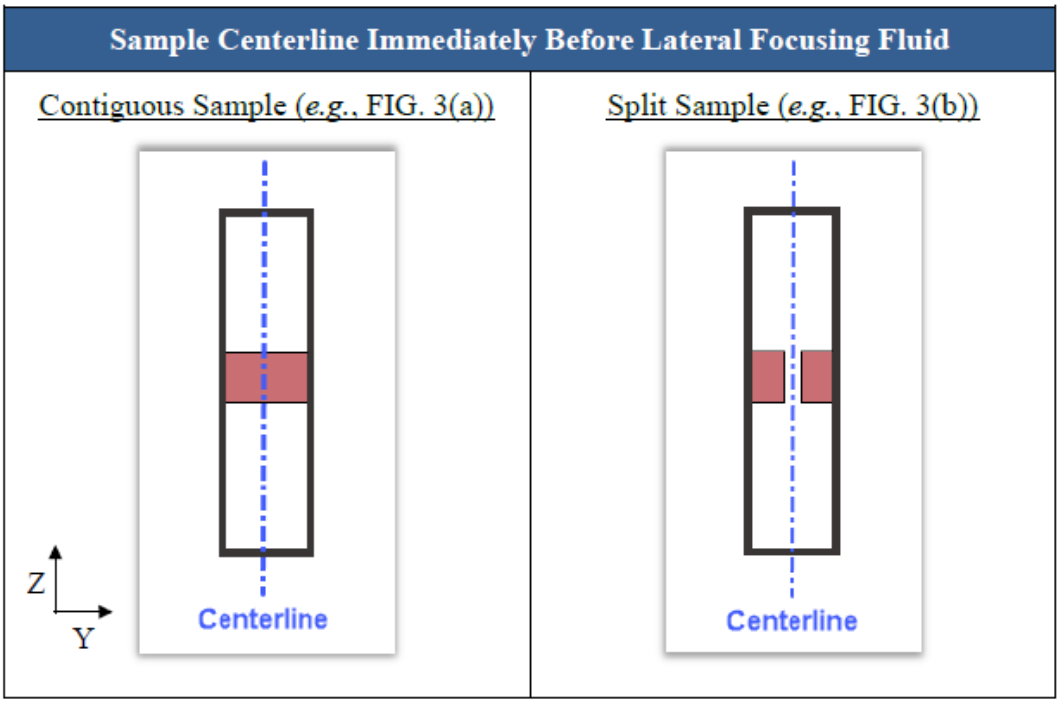
We recognize Patent Owner's arguments that increasing the flow rate may cause split streams to drift further apart and that this would not be "focusing." PO Resp. 10 ("when there are multiple sample streams in a channel, increasing the sheath fluid flow rate will cause the sheath fluid to split the sample streams farther apart) (citing Ex. 2006 ¶ 17). However, the claim is silent as to changes in flow rate, and although the burden rests with Petitioner, Patent Owner has not adequately explained why there could not be a narrowing of the sample overall ("focusing") when there is a split stream. We have reviewed paragraph 17 of the Arnold Declaration and although Dr. Arnold explains that in a split stream, the two sample streams move away from the center of the channel, Dr. Arnold does not adequately explain why this would necessarily prevent constricting of the sample overall. We recognize that the flow rate may increase, for example, as a device narrows. *See, e.g.*, Ex. 2006 ¶ 17 (citing Ex. 1031, 166–168). We regard the issue of whether the sample narrows as a factual issue with respect to the application of the claim term "focusing" to each prior art device, rather than a claim construction issue.

As above, we do not agree with Patent Owner that the claims are limited to "sheath flow." However, we are persuaded by Patent Owner's argument that "the sample stream" is singular in this case.

Although we recognize the rule that the article "a" or "an" is presumed to mean one or more absent a clear intent to limit "a" or "an" to one, *Convolve, Inc. v. Compaq Computer Corp.*, 812 F.3d 1313 (Fed. Cir. 2016), and the Specification also states that "a" or "an" means "one or more" (*see* Ex. 1001, 18:27–30), we agree with Patent Owner that such an interpretation would not be consistent with the claim language in this case.[14]

For example, in claim 2, there is "a centerline of the sample stream." We do not agree with Petitioner that there can be a "centerline" of the sample stream in the case of a split sample. Petitioner's contentions are illustrated with further reference to the figures below.

[14] Further, our interpretation is consistent with the plain and ordinary meaning of "the sample stream" as singular. *Cf. Apple Inc. v. MPH Techns. Oy*, Case 28 F.4th 254, 261–262 (Fed. Cir. 2022) (presumption that a plural refers to two or more items is an application of the general rule that claim terms are usually given their ordinary meaning). We do acknowledge that in *Apple*, there was no rule of construction provided in the Specification. *See id.* at 262.



These are figures reproduced from paragraph 34 of Dr. Issadore's Reply Declaration (Ex. 1054), which show where Dr. Issadore would draw a centerline in a contiguous sample and a split sample, e.g., in a cross section of Simonnet's device immediately before the symmetrical introduction of sheath from the sides, and where the outer black represents the channel walls. Ex. 1054 ¶ 34. Dr. Issadore opines, in the context of the prior art, that when the sample is split, the centerline of the stream is in the middle of the flow channel. Ex. 1054 ¶ 12 (citing Ex. 1003 ¶ 194). Dr. Issadore also opines that a person of ordinary skill in the art would simply draw a centerline through the center of the device where the sample enters, which in Simonnet in through port B. *See* Ex. 1054 ¶ 36. However, the claim refers to the centerline of the sample stream, and not simply the center of the device. [15]

[15] We note that Dr. Issadore does not provide a basis for his opinion that the centerline of the sample stream would be the centerline of the flow channel

Although dependent claims 2 and 23 recite a "centerline of the sample stream," by contrast dependent claim 5 recites "a centerline of the flow channel" and dependent claim 20 recites "a centerline of the microfluidic channel." This difference in language indicates that the "centerline of the sample stream" is different than the "centerline of the flow channel" or "centerline of the microfluidic channel." Dr. Issadore's testimony is more consistent with dependent claims 5 and 20 than dependent claim 2 (at issue here) or dependent claim 23.[16]

Dr. Issadore also opines that in the case of a split stream (in the context of the prior art), the centerline would be through a gap between the streams, in which case the drawn centerline runs through the sheath fluid (instead of the sample). *See* Ex. 1054 ¶¶ 33–34; Ex. 2009, 401:23–402:3. We determine that this would not be a centerline of the sample stream because it runs through sheath fluid. Even if this could be considered a centerline of the sample, it is not a centerline of the sample stream.

We observe that "the sample stream" in the phrase "a centerline of the sample stream" in claim 2 (Ex. 1001, 18:67) refers back, as a matter of antecedent basis, to "a sample stream" in claim 1, i.e., "an inlet configured

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or the centerline of the device at the point where the sample enters. Paragraph 12 of Exhibit 1054 refers to paragraph 194 of Exhibit 1003, where Dr. Issadore draws such a line, but Dr. Issadore does not provide underlying evidence for his opinion that this would have been the understanding of a person of ordinary skill in the art. Patent Owner challenges this interpretation for the situation where there is a split sample, which we discuss below.

[16] The Specification refers, *inter alia*, to the "centerline CL of the core stream forming geometry 400" (Ex. 1001, 10:14–17) and the "center of the lateral fluid focusing channel 520" (Ex. 1001, 15:6–7), which is consistent with dependent claims 5 and 20 rather than dependent claims 2 and 23.

to receive a sample stream" (Ex. 1001, 18:48). One might argue that the sample stream is limited to the sample that enters the device, and such an interpretation would on its face be consistent with Dr. Issadore's opinion that a person of ordinary skill would understand the centerline to be a centerline of the device where the sample enters. *See* Ex. 1054 ¶ 36. However, the claims use the term "stream" to refer to the entire stream that flows through the device, and not simply the portion of the stream that enters the device. For example, claim 1 refers to a plane that is "upstream" and a plane that is "downstream." *See* Ex. 1001, 18:57–61 ("wherein a bottom surface of the flow channel lies in a first plane upstream of the first and second vertical fluid focusing features and the bottom surface of the flow channel shifts vertically upward to lie in a second plane downstream of the first and second vertical focusing features"). The Specification is also consistent with this understanding of the sample stream as the particles of sample flowing through the device (as opposed to only the sample that enters the device). *See* Ex. 1001, 11:13 ("both of which may contribute to shaping sample stream S"), 12:1–3 ("As the sample stream and the focusing fluid progress along the lateral fluid focusing chamber **420**"), 13:7–9 ("A first downwards influence on the sample stream is created upon entry into the lateral fluid focusing chamber **420**"), 14:17 ("Thus, after being laterally focused, the stream is vertically focused downward and then vertically

focused upward"), 14:33 ("The focused stream exits the fluid focusing region **530** in the P2 plane").[17] [18]

Further, if the term "stream" were plural such that there are two streams, then the term "centerline" might also be plural with each stream having its own centerline, in which case it would not be possible to "introduce fluid into the flow channel symmetrically with respect to a centerline of the sample stream," as claimed in claim 2. *See* Ex. 2006 ¶ 62 (opining that each stream would have its own centerline).

This case resembles *Wonderland Nurserygoods Co., Ltd. v. Baby Trend, Inc.*, 727 F. App'x 1017, 1019 (Fed. Cir. 2018), where the ordinary language of the claims indicated that the terms "a fabric member" and "an enclosure member" were singular. In that case, a plural reading of "fabric member" or "enclosure member" would have erased a meaningful distinction between "side panels" and these other terms. *Id.* Further, the "fabric member" and "enclosure member" had to be unitary structures capable of delineating or surrounding an enclosed space. *Id.* Similarly, in this case, a plural interpretation of "stream" would be inconsistent with the claim language, e.g., because it might not be possible to draw a centerline of a sample stream and to introduce focusing fluid symmetrically with respect

[17] In one instance, the Specification states: "There may be certain efficiencies gained in several stream lined aspects relating to the sheath fluid flow illustrated in FIGS. 5A-5D." Ex. 1001, 15:57–60. However, we do not understand this to require several streams but merely to refer to stream-lined aspects where the *aspects* in *several* figures are referred to in the plural. Also, the Specification refers to stream forming geometries in the plural. *See* Ex. 1001, 18:3.

[18] In multiple places the Specification also refers to a "core stream" or "core stream forming geometry." Ex. 1001, *passim*.

to a centerline of the sample stream when there is more than one stream (i.e., in the case of a split sample).

Therefore, we construe "the sample stream" in the claim phrase "configured to focus the sample stream" to refer to a singular stream.

c) Conclusion as to "focusing" and "configured to focus the sample stream"

We maintain our construction of "focusing" as "pinching, confining, squeezing, or constricting" and decline to import additional requirements such as sheath flow into the term "focusing." Nevertheless, we construe "the sample stream" in the claim phrase "configured to focus the sample stream" to refer to a singular stream.

2. "fluid focusing region"

In the Decision on Institution, we set forth our preliminary construction of the focusing region to mean "a portion of the microfluidic unit in which fluid is focused." Dec. Inst. 21.

Patent Owner argues that the Board's construction of "fluid focusing region" should be revised to "a region where focusing is achieved and maintained." PO Resp. 12. Patent Owner argues that if the "fluid focusing region" did not need to achieve and maintain focusing throughout the region, the term would serve no purpose in the claims such that it would read words out of the claim and would lead to an absurd result. *See id.* at 12–13 (citing *Info-Hold, Inc. v. Muzak LLC*, 783 F.3d 1365, 1374 (Fed. Cir. 2015); *AIA Engineering Ltd. v. Magotteaux Intern. S/A*, 657 F.3d 1264, 1276 (Fed. Cir. 2011); Ex. 2006 ¶ 70). Patent Owner also argues that if a "fluid focusing region" needed only to achieve some focusing somewhere in it, regardless of subsequent widening or defocusing, it would be redundant of the term "fluid focusing features." *Id.* at 12 (citing Ex. 2006 ¶ 69). Patent Owner argues

that a "fluid focusing region" cannot defocus. PO Sur-reply 7. Patent Owner argues that each vertical fluid focusing region in Figure 1 of Kim ends when the channel widens. PO Resp. 14 (citing Ex. 2006 ¶¶ 72–74).

Petitioner replies, *inter alia*, that "fluid focusing region" is not redundant over "fluid focusing features" because the "fluid focusing features" are the components used to achieve focusing within the "fluid focusing region." Pet. Reply 8. Petitioner argues that the region's purpose of focusing is satisfied if the sample flow arrives at the inspection region with the desired characteristics. *Id.* Petitioner argues that in Kim, the vertical focusing regions include the portion of the channel that widens after the introduction of fluid. *Id.* at 9 (citing Ex. 1054 ¶ 16, Fig. 1). Petitioner's contentions are illustrated with further reference to the figures below.

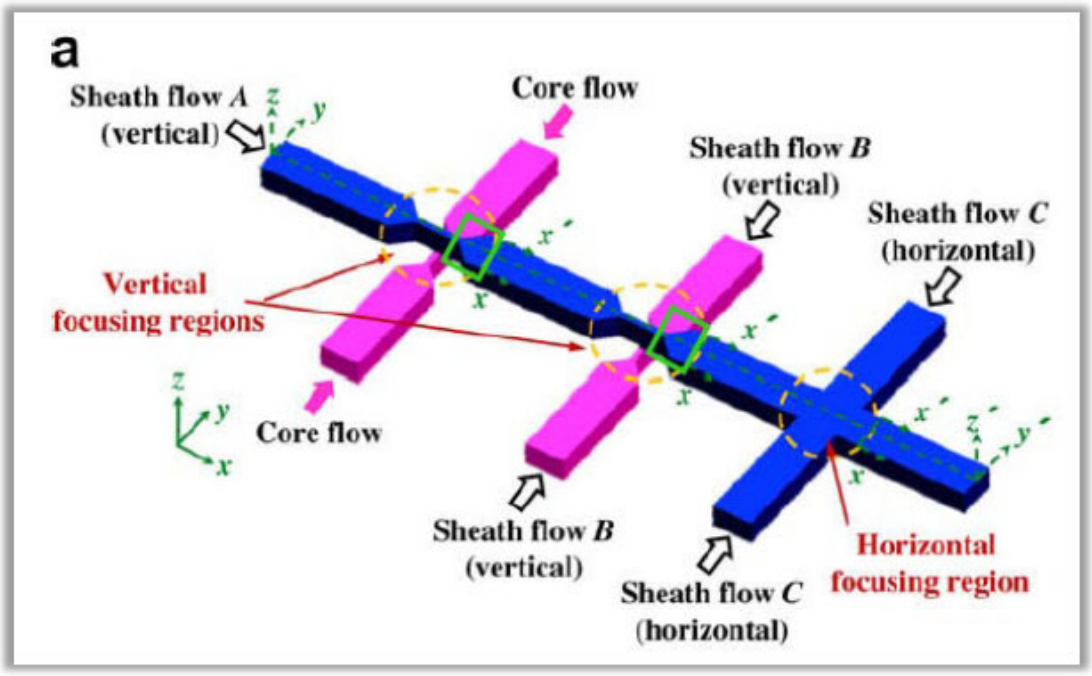


Figure 1 is a schematic diagram of the three-dimensional hydrodynamic focusing device proposed in Kim with an overall view (a). Ex. 1015, 1344. The above annotated figure from Dr. Issadore's Reply Declaration shows Figure 1 of Kim with green rectangle annotations added, i.e., to show areas

where the sample stream widens and which Dr. Issadore avers to be part of the vertical focusing region. *See* Ex. 1054 ¶ 16.

Petitioner also argues, in the context of the prior art, that Patent Owner's arguments depend on the assumption that focusing must be achieved and maintained in all directions at once, which is in tension with the claim requirement that the lateral and vertical fluid focusing features be located at "different longitudinal locations along the flow channel." Pet. Reply 20 (citing Ex. 1054 ¶ 32). Petitioner also argues that even if the sample flow widens (e.g., in the horizontal direction during the process of horizontal focusing), vertical focusing that has been achieved may be maintained. *Id.* (citing Ex. 1054 ¶ 31).

Upon review of the arguments and evidence presented during the trial, we maintain our construction of "fluid focusing region" as "a portion of the microfluidic unit in which fluid is focused." We agree with Petitioner that the claimed "fluid focusing region" is composed of a horizontal fluid focusing region and two vertical fluid focusing regions and therefore the term "fluid focusing region" is not redundant over the component "fluid focusing features." We are also persuaded by Petitioner that the focusing may be achieved or maintained in one direction while being loosened in another direction, as long as by the end of the fluid focusing region, focusing has been achieved in both the horizontal and vertical directions.

We agree with Dr. Issadore that Kim supports the notion that a focusing region may contain areas of widening (designated in Dr. Issadore's Figure as green rectangles, Ex. 1054 ¶ 16) within the yellow circles marked in Kim as vertical focusing regions (*see* Ex. 1015, 1344 & Fig. 1).

Further, based on the claim language, we understand the claimed "fluid focusing region" to provide an output of focused sample fluid to the

claimed "inspection region." The "fluid focusing region" is "configured to focus the sample stream." Ex. 1001, 18:49. Thus, "focusing" is the objective for which the region is configured, which is designed to be accomplished by the action of the three "fluid focusing features." *Cf. Aspex Eyewear Inc. v. Marchon Eyewear, Inc.*, 672 F.3d 1335, 1349 (Fed. Cir. 2012) ("the phrase 'adapted to' is most naturally understood to mean that the arms and magnetic members are designed or configured to accomplish the specified objective, not simply that they can be made to serve that purpose."). Thus, although the claim does not simply encompass any region that is merely capable of focusing and requires focusing features that are designed to focus, the claim does not contain a limitation of continuous narrowing within the region.

3. Additional claim construction issues addressed in post-hearing briefing

We address the additional issues together to the extent necessary to resolve the present dispute.

The key passage in the Specification is as follows:

> As used herein the terms "vertical," "lateral," "top," "bottom," "above", "below," "up," "down," and other similar phrases should be understood as descriptive terms providing general relationship between depicted features in the figures and not limiting on the claims, especially relating to flow channels and microfluidic chips described herein, which may be operated in any orientation.

Ex. 1001, 6:4–10.

Petitioner argues that this passage is definitional and is lexicography by the applicant in the Specification. *See* Pet. Supp. Br. 2–5. Petitioner asserts that this language shows that the terms referenced in the passage are intended to be relative terms. Patent Owner responds, *inter alia*, that the parties agree that claim 1 is not limited to one orientation, provided that the

components of the microfluidic chip are positioned relative to one another as claimed, i.e., in view of the cited passage from the Specification. PO Supp. Br. 2–5. We agree with Petitioner that the applicant in the Specification was acting as its own lexicographer with the language "as used herein," and that the terms "bottom" and "up" are relative such that the claimed device can be in any orientation. We need not reach any further claim construction issue on the facts of this case.

D. Anticipation of Claims 1, 2, 6, and 8 over Simonnet

Petitioner contends that claims 1, 2, 6, and 8 are anticipated by Simonnet. Pet. 29–62. Patent Owner disagrees. *See* PO Resp. 18–31.

1. Simonnet

Simonnet is an article which describes a design, fabrication, and operation of two types of flow cytometers based on microfluidic devices, and presents some experimental results obtained therewith. *See* Ex. 1005, 5653.[19]

Simonnet discloses a molded microfluidic chip that allows tight hydrodynamic focusing in both in-plane and out-of-plane directions, i.e., three dimensional focusing. *Id.* at 5654. Simonnet discloses devices made of a single cast of PDMS sealed with a cover glass, which are disposable and compatible with standard short-working-distance microscope objectives. *Id.* Figure 1 depicting Simonnet's high-throughput device is reproduced below:

[19] Petitioner appears to rely on the first type of device described in Simonnet, which is a high-throughput device. *See* Pet. 23–24.

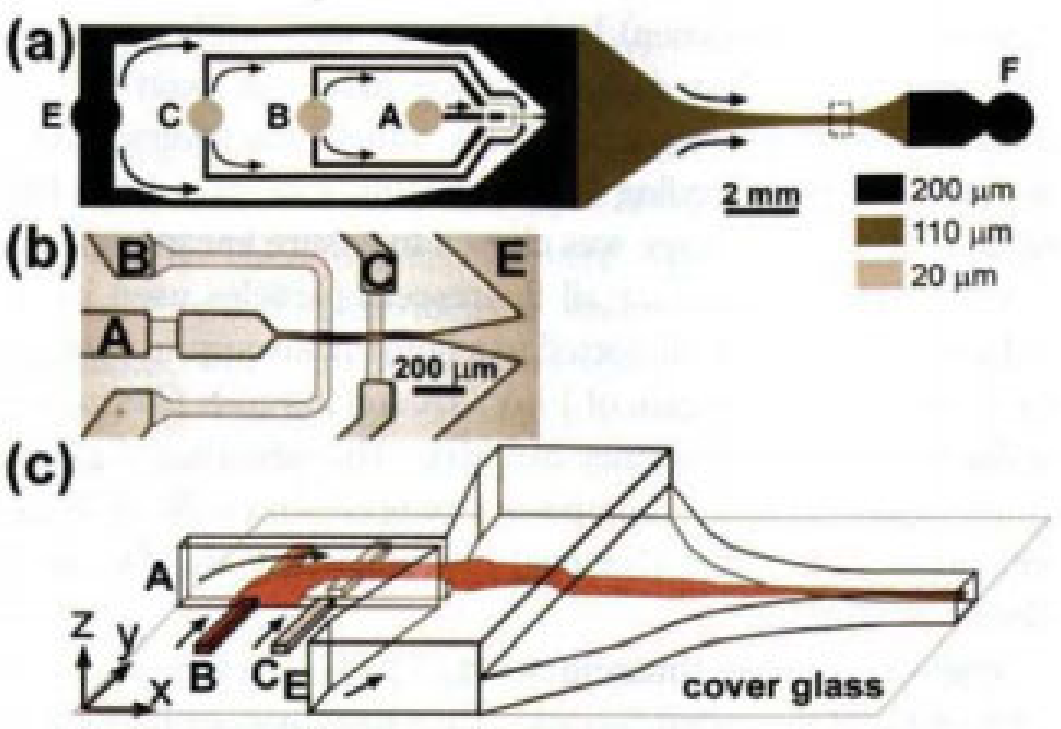


Figure 1 of Simonnet depicts a high-throughput device with arrows showing the direction of flow. Ex. 1005, 5655. A suspension of particles (the sample) is injected into port B. *Id.* Flow focusing in the out-of-plane (z) direction is provided by the liquids injected into ports A (focusing from the top) and C (focusing from the bottom). *Id.* Flow focusing in the in-plane direction is provided by the liquid injected into port E. *Id.* Port F is the device outlet. *Id.* The dashed line box on the top right of the figure indicates the cytometry channel. *See id.*

2. Analysis of Independent Claim 1

a) Preamble: "[a] microfluidic assembly for use with a particle processing instrument, the microfluidic assembly comprising";

b) "a substrate";

c) flow channel: "a flow channel formed in the substrate";

d) sample stream inlet: "the flow channel having: an inlet configured to receive a sample stream"

Petitioner argues, *inter alia*, that Simonnet describes a microfluidic device having an assembly formed by multiple layers of materials and used to focus a stream of particles and/or to image the focused particle stream in an interrogation region. Pet. 29–30 (citing Ex. 1005, 5653–5654, 5656–5657; Ex. 1003 ¶ 126). Petitioner argues that Simonnet discloses a "substrate" because its microfluidic chips are made of "PDMS"—a plastic-based material in which channels are formed—and a "cover glass" used to enclose such channels and which serves as the bottom or top boundary of the microchannels formed in the chip. Pet. 30 (citing Ex. 1005, 5653–5655; Ex. 1003 ¶ 131). Petitioner argues that Figure 1(a) of Simonnet shows numerous channels into which fluids are introduced at inlets ports A, B, C, and E, and that there is a primary channel that permits the flow of fluid from the sample inlet (at port B) along the direction labeled "X" in Figure 1(c). Pet. 31–33 (citing Ex. 1005, 5654; Ex. 1003 ¶¶ 134, 140–141).

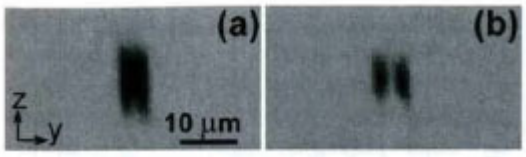
Patent Owner does not separately dispute Petitioner's contentions as to these recitations. *See* PO Resp. 18–31. We accept Petitioner's undisputed

assertions as factual findings. *See generally In re NuVasive*, 841 F.3d at 974.[20] [21]

> *e) fluid focusing region: "fluid focusing region configured to focus the sample stream";*
>
> *f) fluid focusing features: "the fluid focusing region having a lateral fluid focusing feature, a first vertical fluid focusing feature, and a second vertical fluid focusing feature"*

For these limitations, Patent Owner disputes whether "focusing" occurs for Petitioner's asserted fluid focusing features (and whether there is a single stream) and also disputes whether the asserted fluid focusing features are arrayed in a single fluid focusing region as required. PO Resp. 19–27.

As context, the parties disagree on the interpretation of Figures 3(a) and 3(b) of Simonnet, reproduced below:



Figures 3(a) and 3(b) are confocal micrographs showing the flow structure near the central axis of the cytometry channel in HTD (the High throughput device depicted in Figure 1), at conditions typical for cytometry assays,

[20] Although Simonnet's device receives a sample stream, the sample subsequently splits as it flows through the device. We note that below Patent Owner disputes the limitation "focus the sample stream" because the sample splits and does not remain a unitary sample stream.
[21] Regardless of whether the preamble is limiting, Petitioner has shown that the recitation in the preamble is satisfied by the prior art. *See Allen Eng'g Corp. v. Bartell Indus., Inc.*, 299 F.3d 1336, 1346 (Fed. Cir. 2002) ("Generally, the preamble does not limit the claims.").

where dark areas correspond to the stream from port B. *See* Ex. 1005, 5657 (caption to Figure 3); *see also id.*, 5655 (caption to Figure 1).

Petitioner argues that sheath flows from ports A, C, and E all contribute to focusing sample flow from port B. Pet. 35–36 (citing Ex. 1005, 5652, 5654–5655, 5657, Fig. 3; Ex. 1003 ¶ 146). Petitioner argues, with respect to the fluid focusing features, that when the flow of particles reaches the downstream cytometry channel, the flow is confined to a width of 5 μm (or 5–10 μm). *Id.* at 38 (citing, e.g., Ex. 1005, 5654, 5657, 5662, Fig. 3; Ex. 1003 ¶ 151). Petitioner relies on statements in Simonnet describing "focusing" (citing Ex. 1005, 5656–5660) and statements in other publications stating that Simonnet focused a sample (citing Ex. 1046, 1875; Ex. 1047, 3155; Ex. 1006, 973; Ex. 1018, 621; Ex. 1048, 3035; Ex. 1003 ¶ 59; Ex. 1013, 250; Ex. 1049, 120–121, Ex. 1051, 319–320). Pet. Reply 11–12.

Patent Owner argues that Simonnet's device does not focus the sample stream because the resulting sample stream is split. PO Resp. 19–25. Patent Owner asserts that although Simonnet's Figure 1 does not depict the sample stream in any detail, the accompanying text indicates that "the [sample] stream from inlet B is split into two parts, with a gap in the middle." PO Resp. 20 (citing Ex. 1005, 5657). Patent Owner asserts that Figure 3(b) is an image of a cross-section of the Figure 1 device downstream of the alleged "fluid focusing region," and it depicts two sample streams flowing side-by-side, divided by sheath fluid. PO Resp. 20 (citing Ex. 2001 ¶¶ 134–135; Ex. 2006 ¶ 76). Patent Owner argues that Simonnet also discloses another cross section of the sample streams in Figure 3(a), for which Simonnet admits a "residual gap can also be seen." Ex. 1005, 5657.

Petitioner argues that Figure 3(a) depicts a single, contiguous, focused sample. Pet. Reply 14. Petitioner argues that Simonnet distinguishes Figure 3(b)'s "gap in the middle" from Figure 3(a)'s "residual gap," which skilled artisans would understand to refer to the cross section shape of 3(a)'s sample: it has a "notch" in the bottom but is not split through the middle. *Id.* (citing Ex. 1054 ¶ 18).

Petitioner also argues that the "gap" between streams in Figure 3(b) is much smaller than the diameter of a typical mammalian cell, so that cells would be expected to flow single-file, and were associated with the highest accuracy. Pet. Reply 13 n.6 (citing Ex. 1054 ¶¶ 21, 27).

Patent Owner argues that contrary to Petitioner's argument, Simonnet's experiments did not use 10-μm mammalian cells, but rather used much smaller beads instead. PO Sur-reply 12 n.2 (citing Ex. 1005, 5657).

We agree with Patent Owner that Simonnet discloses split sample streams because Simonnet discloses that the stream of Figure 3(b) is "split into two parts, with a gap in the middle" and "[a] residual gap can also be seen in Figure 3a." Ex. 1005, 5657.

Petitioner argues that Simonnet still discloses a device "configured to" focus the sample stream because an apparatus claim covers what a device is and not what it does. Pet. Reply 14–15 (citing *Paragon Solutions, LLC v. Timex Corp.*, 566 F.3d 1075, 1090 (Fed. Cir. 2009)). Apparently referring to Figure 3(a), Petitioner argues that the so-called "gap" occurs only for certain ratios of flow rates between channel A (first sheath) and channel B (sample). *Id.* at 14 (citing Ex. 1005, 5657). Petitioner argues that Simonnet cites

earlier work in Simonnet 2005[22] (Ex. 1005, 5657 (citing Ex. 1020), which shows how varying flow-rate ratios can produce or avoid a gap. Pet. Reply 14 (citing Ex. 1020, 114104-2; Ex. 1014, 1483 & Fig. 7(a)). Petitioner argues that skilled artisans reading Simonnet alone would understand that Simonnet discloses a device configured to produce a "single sample with sheath flow" under a wide range of conditions, even if there are some conditions that will lead to a split sample. *Id.* at 15 (citing Ex. 1054 ¶ 19).

Patent Owner replies that Simonnet makes only a brief citation to Simonnet 2005 and does not specifically or expressly incorporate any part of that paper. PO Sur-reply 13 n.3. Patent Owner cites *Callaway Golf* for the proposition that "[m]ere reference to another . . . publication is not an incorporation of anything therein." *Id.* (citing *Callaway Golf Co. v. Acushnet Co.*, 576 F.3d 1331, 1346 (Fed. Cir. 2009)).

We agree with Patent Owner that Petitioner has not adequately shown that Simonnet would have been understood to incorporate Simonnet 2005 for purposes of an anticipation analysis. In particular, Petitioner has not shown that the device in Simonnet 2005 is the same device, nor adequately explained how the teachings of Simonnet 2005 would apply to the device of Simonnet. We agree with Patent Owner that Simonnet merely refers to Simonnet 2005 without clearly identifying material that is incorporated. *See Callaway Golf Co.*, 576 F.3d at 1346. Indeed, as relevant to Petitioner's contentions, Simonnet 2005 is used in a footnote as a citation for the proposition that the gap occurs when the flow emerging from the channels B is small compared with the flow in channel A, and the streams from

[22] Simonnet, C.: Groisman, *Two-dimensional hydrodynamic focusing in a simple microfluidic device*, 87 APPL. PHYS. LETT. 114104 (2005) (Ex. 1020, "Simonnet 2005").

channels B do not reach the middle of channel A. Ex. 1005, 5657. Petitioner has not shown that Simonnet would incorporate an embodiment from Simonnet 2005 with no gap.

Applying our claim construction which requires a single sample stream, we determine that Simonnet's split sample does not satisfy "the sample stream" limitation.[23]

"fluid focusing region"

Petitioner sets forth its contentions that Simonnet discloses a fluid focusing region with a lateral fluid focusing region and two vertical fluid focusing regions. Pet. 37–50. Patent Owner disputes that Simonnet discloses a single fluid focusing region because, according to Patent Owner, Simonnet discloses a widening in the lateral fluid focusing feature. PO Resp. 2, 26. To provide context, we first reproduce Petitioner's contentions for the three fluid focusing features as follows.

For a first vertical focusing feature, Petitioner argues that Simonnet discloses that inlet port A introduces focusing fluid into the flow channel of the device, which narrows or confines the sample in a vertical direction. Pet.

[23] In addition, Petitioner argues in a footnote in its Reply brief that mammalian cells could be used, i.e., in Simonnet's system in place of beads, and citing Dr. Issadore's Reply Declaration. Pet. Reply 13 n.6 (". . . and the 'gap' between streams is much smaller than the diameter of a typical mammalian cell, so that cells would be expected to flow single-file") (citing Ex. 1054 ¶ 21). Although we address this argument in more detail in the obviousness section below, we also address this argument as an argument for anticipation as follows. Simonnet suggests that cells can be used (*see* Ex. 1005, 5662), but does not disclose a single stream of cells. Dr. Issadore avers that cells would not "typically" arrive at the inspection region at once (i.e., side by side) (Ex. 1054 ¶ 21), but this stops short of a statement that there is a single stream.

38 (citing Ex. 1005, 5654). Petitioner's contentions are illustrated with further reference to the annotated figure below.

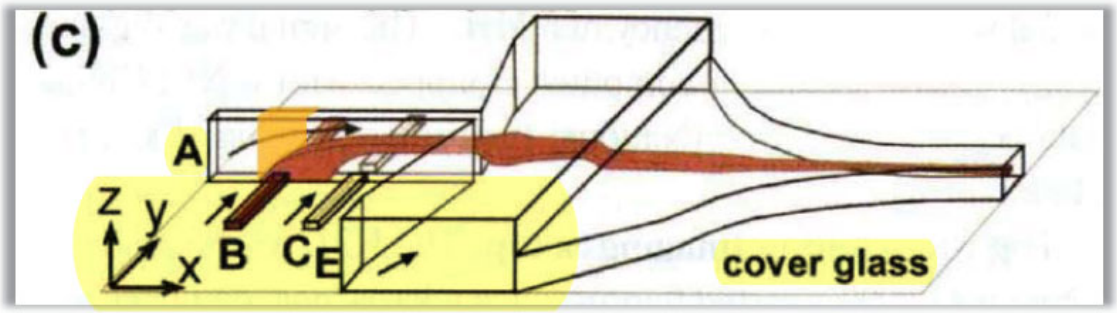


Figure 1(c) of Simonnet annotated by Petitioner is provided above, with yellow and orange color added. *Id.* at 39. Petitioner asserts that this figure illustrates that focusing fluid from port A enters the flow channel and pushes vertically downward against the sample flow from port B to focus that fluid from the top. *Id.* at 39 (citing Ex. 1005, 5652, 5657, 5662; Ex. 1003 ¶ 152).

Petitioner asserts that the vertical focusing is confirmed in Simonnet's experimental results, which show that the sample is confined to a cross section of 5–10 μm near the central axis of a cytometry channel. *Id.* (citing Ex. 1005, 5654, 5657, 5662, Fig. 3).

For a second vertical focusing feature, Petitioner argues that Simonnet discloses that the pair of sheath fluid inlets (at C) introduces focusing fluid into the flow channel of the device, to focus the sample in the vertical direction. Pet. 39–40 (citing Ex. 1005, 5654). Petitioner argues that Simonnet discloses that "flow focusing in the out-of-plane (z) direction is provided by the liquid[] injected into port[] … C (focusing from the bottom)." *Id.* at 40 (citing Ex. 1005, Fig. 1). Petitioner's contentions are illustrated with further reference to the annotated figure below.

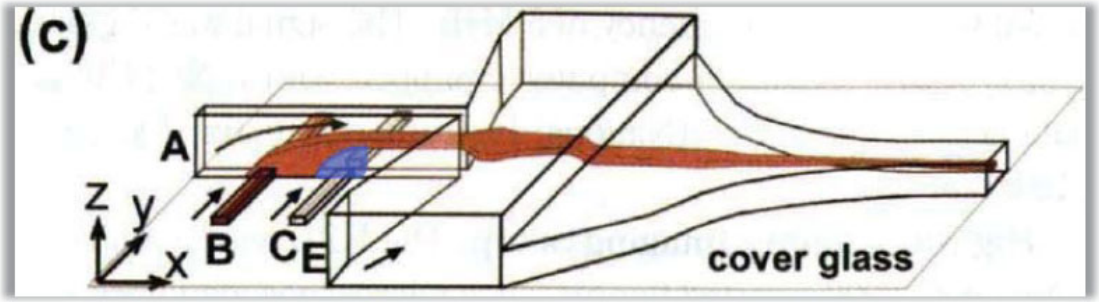


Figure 1(c) of Simonnet annotated by Petitioner, is provided above, with blue color added. *Id.* at 40. Petitioner argues that Simonnet's experimental results show that the sample is confined to a cross section of "5-10 µm near the central axis of a cytometry channel, which has a cross section of 300 x 100 µm." *Id.* (citing, e.g., Ex. 1005, 5654). Petitioner argues that focusing fluid from port C helps to confine the sample to near the center of the channel by pushing upward on the sample from below. *Id.* at 40–41 (citing Ex. 1003 ¶ 153).

For a lateral fluid focusing feature, Petitioner argues that Simonnet's device includes a component that introduces sheath fluid (a focusing fluid) through the channels labeled "E" to narrow or constrain the sample in a lateral direction (corresponding to the "y" direction in the axis shown on FIG. 1(c), illustrated with arrows below). Pet. 37 (citing Ex. 1005, Fig. 1).

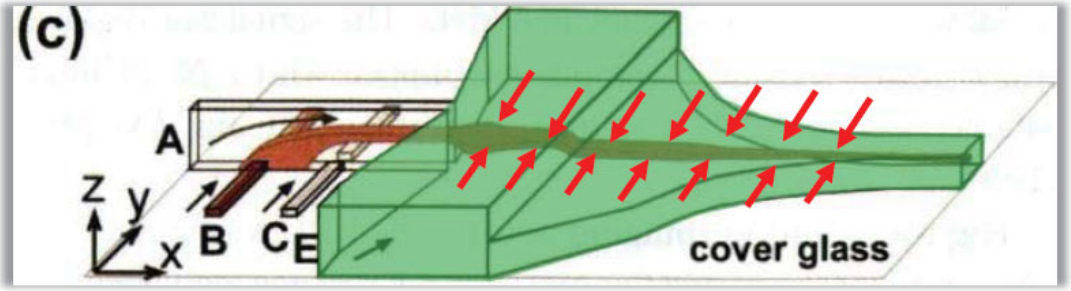


Figure 1(c) of Simonnet annotated by Petitioner is reproduced above, with green color and red arrows added. *Id.* Petitioner asserts that Simonnet discloses that "[f]low-focusing in the plane of the device (in the y-direction)

is achieved by squeezing the stream carrying particles between the streams emerging from the two channels, E." *Id.* (quoting Ex. 1005, 5656; citing Ex. 1005, 5652, 5654, 5657).[24]

Patent Owner argues that Petitioner fails to establish that Simonnet discloses a "fluid focusing region" because, in the alleged region, the sample stream "plumes" and thus fails to maintain focusing. *See* PO Resp. 25. Patent Owner's contentions are illustrated with further reference to the annotated figure below.

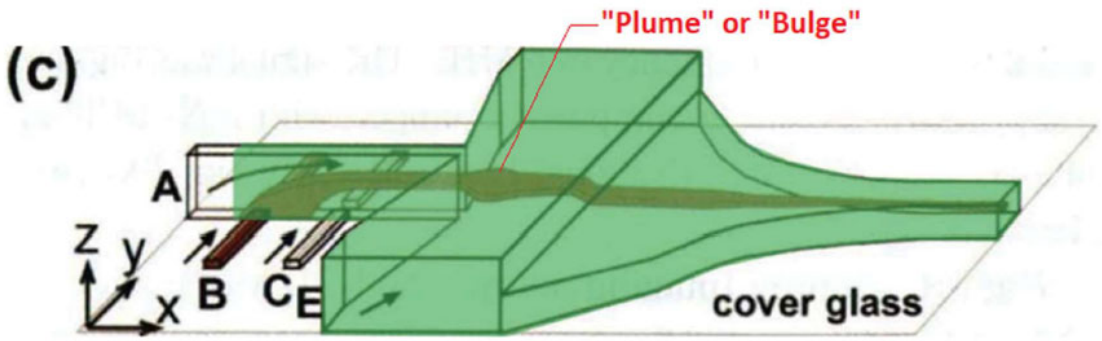


Figure 1(c) of Simonnet annotated by Patent Owner, is provided above, with green color added and a label for part of the sample as "Plume" or "Bulge." *Id.* at 26. Patent Owner argues that while Dr. Issadore refers to the "plume" as a "slight bulge" (Ex. 2008, 175:22–55), Dr. Arnold fully analyzed the device and concluded that the sample stream widens by about eight times its original width at that location. *Id.* (citing Ex. 2001 ¶¶ 122–127, 147–148).

[24] Although the Petition identifies certain alternative parts of Simonnet's device as potential fluid focusing features (*see* Pet. 49–50), counsel for Petitioner represented at the hearing that it is not necessary for the Board to evaluate these alternative theories "because patent owner hasn't disputed that each of the identified features in the primary theory, if the device focuses at all, is a fluid focusing feature." Tr. 5:13–17.

Beyond disputing that the fluid focusing features combine in one fluid focusing region (and otherwise disputing the limitations of "focusing" and "the sample stream"), Patent Owner does not dispute that Simonnet discloses a lateral fluid focusing feature and two vertical fluid focusing features.

Petitioner argues that the lateral fluid focusing region would be part of one fluid focusing region with the vertical fluid focusing features under the Board's claim construction of fluid focusing region as "the portion in which fluid is pinched, confined, squeezed, or constricted." Pet. Reply 19–20. Petitioner also argues that, even under Patent Owner's proposed construction, skilled artisans would readily understand that, if the sample flow "widens" or "plumes," the vertical focusing that had been "achieved" upstream would be "maintained," because the channel remains the same height as it widens laterally. *Id.* at 20 (citing Ex. 1054 ¶ 31).

Applying our claim construction for "fluid focusing region," we determine that the Simonnet's two vertical fluid focusing features and lateral focusing feature are part of a fluid focusing region because the sample is "squeezed" and "confined" as a result of its flow through the three portions of Simonnet's microfluidic channel identified by Petitioner. *See* Ex. 1005, 5654 ("The flow of particles is confined to a region with a diameter of 5–10 μm near the central axis of a cytometry channel, which has a cross section of 300 x 110 μm"); 5655 (caption to Figure 1 states that flow focusing occurs); 5656 ("Flow-focusing in the plane of the device (in the y-direction) is achieved by squeezing the stream carrying particles between the streams emerging from the two channels, E"). However, as above and applying our definition of "the sample stream," we determine that Simonnet's fluid focusing region is not "configured to focus the sample stream" because Simonnet does not disclose a single sample stream.

> *g) Different longitudinal locations: "the lateral, the first vertical, and the second vertical fluid focusing features [are] provided at different longitudinal locations along the flow channel"*

Petitioner argues that the '439 patent uses the terms "longitudinal" or "longitudinally" to refer to the direction along which the sample flow travels in the chip. Pet. 51 (citing Ex. 1001, 13:26–30, Fig. 3D).

Petitioner relies on the locations marked below in orange, blue, and green for the recited fluid focusing features (*see* Pet. 51–52). Petitioner's contentions are illustrated with further reference to the annotated figure below.

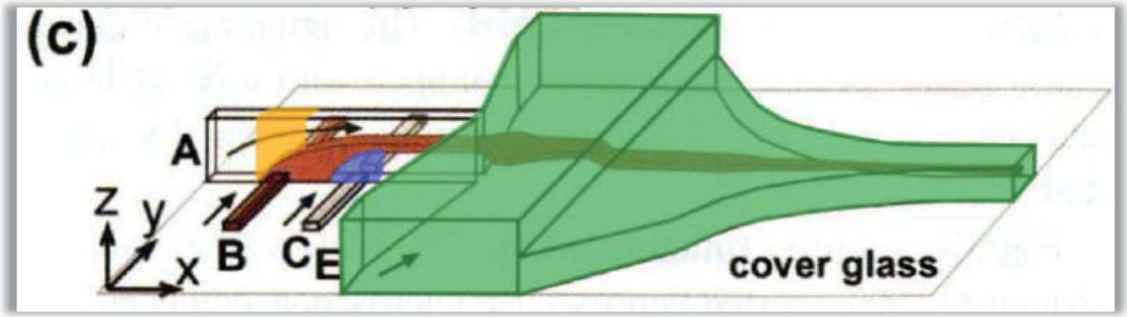


Figure 1(c) of Simonnet annotated by Petitioner, is provided above with color added (*see* Ex. 1005, Fig. 1). *Id.* at 52.

Patent Owner does not separately dispute Petitioner's contentions that the asserted flow focusing features are at different longitudinal locations. *See* PO Resp. 18–31.

We determine that Simonnet's Figure 1(c) demonstrates the recited relationship.

> *h) Upward vertical shift: "wherein a bottom surface of the flow channel lies in a first plane upstream of the first and second vertical fluid focusing features and the bottom surface of the flow channel shifts vertically upward to lie in a second plane downstream of the first and second vertical focusing features"*

Petitioner relies on the vertical shift in the annotated figure (*see* Pet. 54). Petitioner's contentions are illustrated with further reference to the annotated figure below.

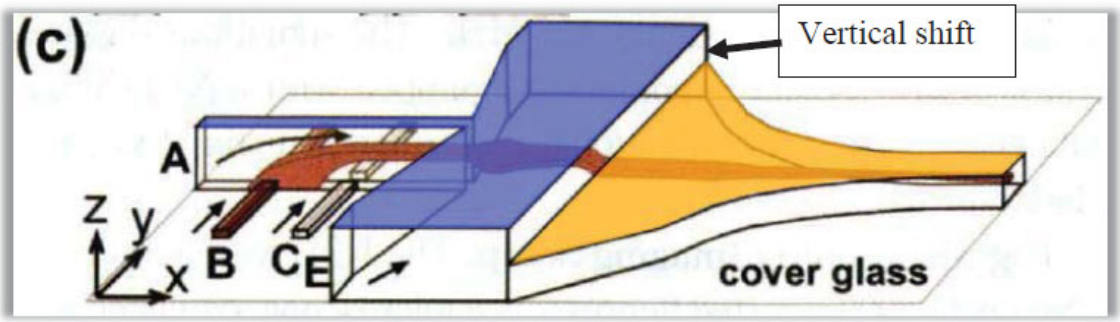


Figure 1(c) of Simonnet annotated by Petitioner, is provided above with color and annotation added (*see* Ex. 1005, Fig. 1). Petitioner asserts that the top height of the device shifts its height downward at the location of the vertical step, i.e., just before the flow channel starts to taper. Pet. 54. However, Petitioner argues that this is equivalent to an upward shift because the '439 patent states that "flow channels . . . may be operated in any orientation" (citing Ex. 1001, 6:8–10) and "bottom . . . should be understood as [a] descriptive term[] providing general relationship between depicted features in the figures and not limiting on the claims" (citing *id.* at 6:4–8). *Id.* at 53–54. According to Petitioner, fluid forces in a chip are stronger than gravity. *Id.* at 54 (citing Ex. 1027, 47). Petitioner argues that a person of ordinary skill would have known that this device could be viewed from (or operated in) a different orientation than that of the image. *Id.* at 55 (citing Ex. 1003 ¶¶ 181–182).

Patent Owner argues that "[a]nticipation requires Simonnet to expressly or inherently disclose each limitation arranged [as] claimed, and the claim expressly requires the 'bottom surface of the flow channel' to 'shift vertically *upward*.'" PO Resp. 27. Patent Owner argues that anticipation by inherency "may not be established by probabilities or possibilities." *Id.* at 28 (citing *Bettcher Indus. v. Bunzl USA, Inc.*, 661 F.3d 629, 639 (Fed. Cir. 2011)). Patent Owner argues that Petitioner must show that Simonnet "necessarily functions in accordance with, or includes, the claimed limitations." *Id.* (citing *Brassica Prot. Prods. LLC v. Sunrise Farms* (*In re Cruciferous Sprout Litig.*), 301 F.3d 1343, 1349 (Fed. Cir. 2002)). Patent Owner argues that Petitioner made no attempt to establish such inherency. *Id.* Patent Owner asserts that Dr. Issadore admitted at his deposition that, though Simonnet "could be operated in a vertically flipped orientation," such vertical flipping would not occur as a "natural result of its normal operation." *Id.* (citing Ex. 2008, 240:11–241:13; Ex. 2006 ¶¶ 100–101).

Petitioner replies that Patent Owner's expert merely testified that the Simonnet chip would not spontaneously flip on its own. Pet. Reply 21 (citing Ex. 2006 ¶ 100). Petitioner argues that Simonnet's device needs no modification, that "top" and "bottom" are relative terms, and that Dr. Issadore testified without contradiction that a skilled artisan looking at Simonnet would recognize that the device "could be analyzed (and operated) in a vertically flipped orientation" relative to what is illustrated in Simonnet Figure 1. Pet. Reply 21–22 (citing Ex. 1003 ¶¶ 179, 181). Petitioner argues that if Patent Owner's contrary understanding were adopted, potential infringers could avoid infringement simply by operating their otherwise-

infringing chips in a different orientation—where a shift "up" is no longer a shift up relative to Earth's surface. *Id.* at 22.

Applying our claim construction of the "wherein" clause, where the terms "bottom surface" and "vertically upwards" are understood to be terms of relative position, we determine that Simonnet's Figure 1(c) both expressly and inherently discloses this limitation of claim 1. In particular, Figure 1(c) depicts a top surface shifting downward, and where these are relative terms, this is the same as a bottom surface shifting upward. Further, as argued elsewhere by Petitioner (*see* Pet. Reply 15), this is a claim for an assembly, not a process, and the device is the same in any orientation regardless of how it is operated.

i) Inspection region: "an inspection region at least partially downstream of the fluid focusing region."

Petitioner argues Simonnet discloses that the flow cytometer design it implements is used to detect "optical properties" of the focused sample particles. Pet. 56 (citing Ex. 1005, 5653). Petitioner argues Simonnet describes "imaging systems" used with its flow cytometry devices, including particle detection, using a camera, microscope, and laser light. *Id.* at 56–57 (citing Ex. 1005, 5654, 5656). Petitioner argues that Simonnet detects particles, which requires inspecting them. *Id.* at 57 (citing Ex. 1003 ¶ 187).

Petitioner also argues that Simonnet refers to a "cytometry channel" as the location where the microscope and camera inspects and detects particles, and that this is downstream of the fluid focusing region. *Id.* (citing Ex. 1005, 5656–5657). Petitioner argues that Simonnet also expressly states that it contains an "interrogation region, where flow cytometry assays were carried out, [] in the central area of the cytometry channel (Figure 1a)" and

argues that this is at least partially downstream of the fluid focusing region. *Id.* at 57–58 (citing Ex. 1005, 5656–5657).

Patent Owner does not separately dispute this limitation. We determine that Simonnet discloses this limitation. Figure 1(a) of Simonnet is depicted below.

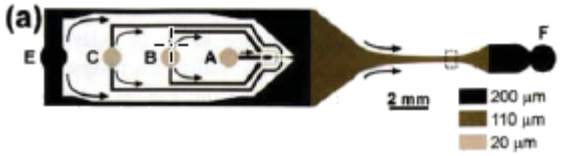


Figure 1(a) of Simonnet is a schematic drawing of the microchannel network, where the dashed line box (on the right) indicates the cytometry channel. *See* Ex. 1005, 5655; Pet. 57. Based on the arrows in Figure 1(a) of Simonnet, the cytometry channel is at least partially downstream of the microfluidic channel. Using confocal microscopy, Simonnet measures fluorescence across the cytometry channel. Ex. 1005, 5656.

j) Summary for claim 1

For the reasons above, we determine that Petitioner has not established that claim 1 is anticipated by Simonnet because Simonnet does not disclose the recited "fluid focusing region configured to focus the sample stream."

3. Analysis of Dependent Claims 2, 6, and 8

Claims 2, 6, and 8 depend from claim 1. Because we find that Petitioner has not established that claim 1 is anticipated by Simonnet, we find that Petitioner has not established that claims 2, 6, and 8 are anticipated by Simonnet for the same reason.

E. Obviousness of Claims 1, 2, 6, and 8 over Simonnet

Petitioner sets forth its contentions that claims 1, 2, 6, and 8 are obvious over Simonnet. Pet. 62–64.

1. Independent claim 1

a) "fluid focusing region configured to focus the sample stream"

Above, we find that Petitioner has not shown that Simonnet anticipates independent claim 1 because Simonnet produces a split sample with more than one stream, which does not qualify as "the sample stream," which we construe to be singular.

Petitioner asserts that Patent Owner has admitted that skilled artisans "would have been encouraged" to follow Simonnet's "suggestion" to "address [the split-stream] issue" by "decreas[ing] the relative flow rate of the sheath fluid." Pet. Reply 15 (citing PO Resp. 33). Petitioner asserts that Simonnet on its face teaches that the "gap" occurs only under certain relative flow conditions (i.e., "when the flow emerging from channels B is small compared with the flow in channel A") (citing Ex. 1005, 5657), and argues that skilled artisans would have readily adjusted those conditions to eliminate any gap, if doing so was desirable for a particular application (citing Ex. 1054 ¶¶ 20–21). Pet. Reply 15–16.[25]

Patent Owner argues that Petitioner has not provided a motivation to combine Simonnet with Simonnet 2005 and has not shown a reasonable expectation of success in doing so. *See* PO Sur-reply 15–20. Patent Owner

[25] Paragraph 20 of the Issadore Reply Declaration tracks Petitioner's statement in this regard and does not add additional reasoning. *See* Ex. 1054 ¶ 20. We address paragraph 21 of the Issadore Reply Declaration in the discussion of mammalian cells below.

argues that Petitioner does not disclose what the other relative flow conditions would be, what particular applications might call for those other flow conditions, nor how or why those undisclosed conditions would meet the other requirements for focusing. PO Sur-reply 16. Patent Owner argues that Petitioner also does not identify any reason or motivation for modifying the Simonnet device to arrive at the claimed invention. *Id.* Patent Owner argues that, if anything, Petitioner is improperly relying on the '439 patent as a blueprint for its obviousness analysis. *See id.* at 16–17 (citing *Orexo AB v. Actavis Elizabeth LLC*, 903 F.3d 1265, 1271 (Fed. Cir. 2018); *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 113 (Fed. Cir. 1985)).

Patent Owner argues that Dr. Issadore admitted in deposition that his opinion only extended to how a person would purportedly avoid splitting the sample stream in Simonnet, not why a person would do so. PO Sur-reply 17–18 (citing Ex. 2009, 345:19–346:7, 340:13–18, 341:8–15). Patent Owner points, *inter alia*, to the following exchange at the deposition:

> [Counsel for Patent Owner] Okay. And -- so they would have known how to do it, but -- in your opinion -- ***but why would they want to eliminate such a split stream?***
>
> [Dr. Issadore] ***I'm not sure I can speak to why, you know, in general an artisan would want to do something.*** Like I said before, there are so many applications of microfluidic, it would really be -- it would really depend on the context of a particular application.

PO Sur-reply 17 (quoting Ex. 2009, 345:19–346:7) (emphasis in brief).

Patent Owner also appears to dispute Petitioner's contention that reducing the flow rate would prevent the sample from splitting. *See* PO Sur-reply 16–17 n.4 (citing PO Resp. 33). Patent Owner also argues that Petitioner's Reply is completely silent on why a person of skill in the art

would have any success in doing so while still achieving the invention claimed in the '439 Patent. PO Sur-Reply 19.

We agree with Patent Owner that Petitioner has not provided adequate reasoning as to why a person of ordinary skill would have sought to modify the teachings of Simonnet, e.g., to vary the flow rate ratios and eliminate the gap in the sample streams. *See* Pet. Reply 14–16. Indeed, Petitioner even states to the contrary that a split sample may be desirable for certain applications. Pet. Reply 5–6 (citing Ex. 1039, 326; Ex. 1054 ¶ 10).[26]

Simonnet does state that "[t]he gap occurs when the flow emerging from channels B is small compared with the flow in channel A" (Ex. 1005, 5657), but Simonnet stops short of suggesting a change in the flow rate ratios and Petitioner and Petitioner's expert do not sufficiently explain why a person of ordinary skill would have sought to decrease the relative flow rate of sheath fluid. *See* Pet. Reply 15. Further, although Petitioner states that Simonnet 2005 "shows how varying flow-rate ratios can produce or avoid a gap," Petitioner has not explained why a person of ordinary skill in the art using the device of Simonnet would have done so. *See* Pet. Reply 14. Dr. Issadore does not provide additional reasoning in the declaration, and as quoted above in the deposition Dr. Issadore was candid that he was not providing reasoning as to "why" a skilled artisan would have wanted to eliminate the split stream. *See* Ex. 1054 ¶¶ 20–21; Ex. 2009, 345:19–346:7, 340:13–18, 341:8–15. Dr. Issadore opines that a person of ordinary skill would have eliminated the gap "if doing so was desirable for a particular application." Ex. 1054 ¶ 20. This statement is conclusory.

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[26] Further, Dr. Issadore states that "the split sample allowed for the most 'accurate' particle detection" (in discussing Dr. Arnold's opinion that "f_d/f_0" is a measure of "accuracy"). Ex. 1054 ¶ 27; *see id.* ¶ 23; *supra* n.13.

As to Petitioner's assertion that Patent Owner has admitted that skilled artisans would have decreased the relative flow rate of the sheath fluid based on a suggestion from Simonnet (Pet. Reply 15 (citing PO Resp. 33)), we disagree. Rather, Patent Owner's argument at page 33 of its Patent Owner Response is that even with a decreased flow rate, a gap would still exist. *See* PO Resp. 33.

Petitioner additionally mentions in a footnote in its Reply brief that mammalian cells could be used, i.e., in Simonnet's system in place of beads, and citing Dr. Issadore's reply declaration. Pet. Reply 13 n.6 (". . . and the 'gap' between streams is much smaller than the diameter of a typical mammalian cell, so that cells would be expected to flow single-file") (citing Ex. 1054 ¶ 21). Patent Owner replies that Petitioner has not provided any evidence to show that a skilled artisan would have any reason to use the same device and flow configuration with mammalian cells or would have had a reasonable expectation of success when using cells that are five times the size of the beads used in Simonnet's experiments. PO Sur-reply 12 n.2 (citing Ex. 1005, 5657 ("we seeded the liquid . . . with 1.9-μm polystyrene beads . . ."); Ex. 2009, 320:23-321:13).

In context, Dr. Issadore avers that if mammalian cells were used, "the focused split sample ***typically*** would not allow two cells to arrive at the inspection region at once, because there is not enough space for two 10-μm cells to travel side by side." *See* Ex. 1054 ¶ 21 (citing Ex. 1005, 5662) (emphasis added). The qualifier "typically" for two cells not arriving at the same time does not indicate that there would be no split stream. And Dr. Issadore seems to concede there could be a split sample. *Id.* Further, Dr. Issadore does not state which dimensions of which of Simonnet's channels

that he is using as a reference.[27] We determine that Dr. Issadore provides insufficient evidence and reasoning for an understanding that there would be a single stream if mammalian cells were used, in addition to making a statement that stops short of that conclusion.

Although not argued by Petitioner nor discussed by Dr. Issadore, Simonnet does state: "we expect the HTD to be compatible with cells up to ~15 μm in size." Ex. 1005, 5662. Simonnet also states: "We expect that with the improvements in optics and detection electronics, a cytometry system based on the HTD would match the throughput, sensitivity, and accuracy of commercial flow cytometers and would be an adequate tool for assaying fluorescently marked live cells." *Id.* These statements can be fairly read as a statement of reasonable expectation of success of using mammalian cells in the device. However, Simonnet is silent as to whether the mammalian cells would be in a single stream, and if anything, Simonnet appears to be inviting further experimentation.

Accordingly, Petitioner has not provided adequate reasoning to modify Simonnet to form a single sample stream.

[27] For example, Simonnet states that "The smallest channels in the HTD (the shallow channels connected to the tall-and-narrow channel; Figure 1b) have a cross section of 60 x 20 μm" (Ex. 1005, 5662) but Simonnet also states that "[t]he 200-μm-deep channels connecting the inlets A, B, and C with the 3D focusing element are 150 μm wide" (Ex. 1005, 5655 (caption to Fig. 1)).

b) "wherein a bottom surface of the flow channel lies in a first plane upstream of the first and second vertical fluid focusing features and the bottom surface of the flow channel shifts vertically upward to lie in a second plane downstream of the first and second vertical focusing features"

Petitioner argues that to the extent that the limitation relating to shifting vertical planes was not met as a matter of anticipation, it would have been obvious to modify the device to meet those limitations, e.g., by flipping the device. Pet. 63 (citing, e.g., Ex. 1003 ¶ 184). Petitioner argues that it would have been obvious to a skilled artisan that Simonnet could be flipped vertically, without changing the characteristics of the device, and would have been a trivial step. *Id.* (citing *Sealy Tech., LLC v. SSB Mfg. Co.*, No. 2019-1872, 2020 WL 5033045, at *4 (Fed. Cir. Aug. 26, 2020); Ex. 1003 ¶¶ 179, 184; Ex. 1001, 6:8–10.

Patent Owner argues that there is no reason why a person of ordinary skill would have modified Simonnet to achieve the "shifts vertically upward" limitation in claim 1. PO Resp. 33–34 (citing *InTouch Techs. Inc. v. VGo Communs. Inc.*, 751 F.3d 1327, 1352–54, n.8 (Fed. Cir. 2014)). Patent Owner argues that the only authority Petitioners cite for side-stepping a reason to modify Simonnet is "an inapposite non-precedential opinion addressing obviousness of a design patent." *Id.* at 34 (citing *Sealy Tech., LLC v. SSB Mfg. Co.*, 825 F. App'x 801, 807 (Fed. Cir. 2020)). Patent Owner argues that in that case, the primary reference had only a trivial difference from the patented design and a similarity in appearance between the primary and secondary references supported a motivation to combine them, whereas here Petitioner fails to explain how such reasoning applies to this ground of obviousness. *Id.*

For the same reasons as for the ground of anticipation by Simonnet, we find that Simonnet expressly and inherently discloses this limitation even without modifying Simonnet's device.

c) Other arguments and conclusion as to claim 1

Petitioner also argues that to the extent the limitation of an inspection region at least partially downstream from a fluid focusing region was not met as a matter of anticipation because fluid is still being focused at the inspection region, it would have been obvious to elongate the device further. Pet. 63–64 (citing, e.g., Ex. 1003 ¶ 191).

Patent Owner argues that Petitioner's arguments do not address the other deficiencies in the ground of anticipation of claim 1. *See* PO Resp. 32.

With respect to the "inspection region," above we find no deficiency in the "inspection region" limitation. However, we determine above that Simonnet fails to disclose "focusing region configured to focus the sample stream," and Petitioner has failed to show that it would have been obvious to modify Simonnet to arrive at this limitation. We, therefore, determine that Petitioner has not established that independent claim 1 would have been obvious over Simonnet.

2. Dependent claims 2, 6, and 8

Because we determine that Petitioner has not established that claim 1 is obvious over Simonnet, we determine that Petitioner has not established that claims 2, 6, and 8 are obvious over Simonnet for the same reason.

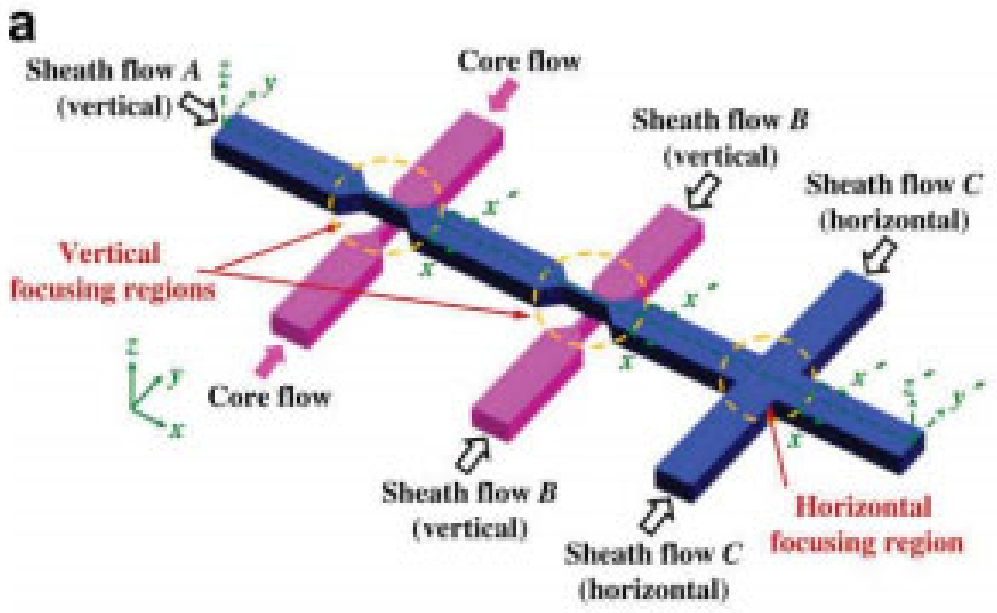
F. Obviousness of Claim 8 over Simonnet and Kim

Petitioner contends that claim 8 would have been obvious over Simonnet and Kim. Pet. 64–66. Patent Owner disagrees. *See* PO. Resp. 35–36.

1. Kim

Kim is an article that describes the author's design, fabrication, and characterization of an efficient three-dimensional hydrodynamic focusing microfluidic device, with experimental results. Ex. 1015, 1343. Kim states that to achieve a high aspect ratio, previous microfluidic devices required extravagantly high channel height (more than 100 μm) or very narrow width, which can develop expensive fabrication costs or increased pressure drop, respectively. *Id.* Kim describes a device where hydrodynamic focusing of core sample flow is effectively achieved in the pressure driven flow owing to a locally increased aspect ratio of thickness to width even below the global aspect ratio of 0.5. *Id.*

Figure 1 of Kim is reproduced below:



b

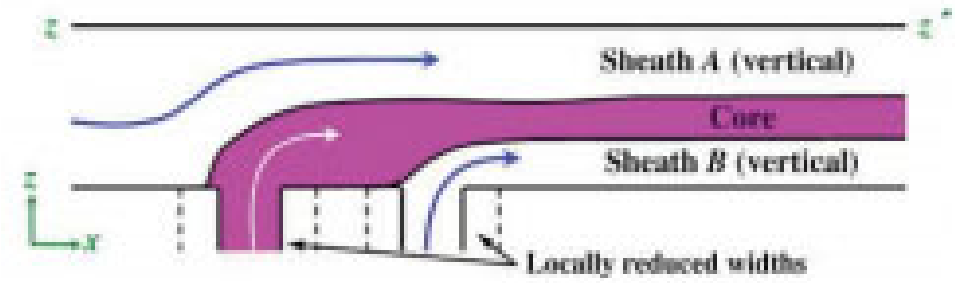


Figure 1 is a schematic diagram of the three-dimensional hydrodynamic focusing device proposed in Kim with an overall view (a) and a vertical view (z-z') (b). Ex. 1015, 1344. The channel width at two vertical focusing regions is locally reduced to 50 μm to achieve the high aspect ratio. *Id.*

2. Analysis of Claim 8

Claim 8 depends from independent claim 1 and further recites "wherein within the fluid focusing region the fluid flow channel transitions from a first cross section shape to a second cross section shape different from the first cross section shape." Ex. 1001, 19:36–38.

Petitioner does not rely on Kim to remedy the deficiency in the ground of obviousness of claim 1 over Simonnet, which we have addressed above. Accordingly, we determine that Petitioner has not established that claim 8 would have been obvious over Simonnet and Kim.

G. Obviousness of Claim 9 over Simonnet and Kummrow

Petitioner contends that claim 9 would have been obvious over Simonnet and Kummrow. Pet. 67–70. Patent Owner disagrees. *See* PO Resp. 37–39.

1. Kummrow

Kummrow is an article that describes three-dimensional microfluidic structures with integrated optical fibers, mirrors, and electrodes for flow cytometric analysis of blood cells, and experimental results obtained therewith. Ex. 1006, 972. Most of the fluidic channels had constant height resulting in the confinement of sample flow in one direction. *Id.* at 973. Kummrow's flow cells feature one inlet for the sheath flow, cascaded hydrodynamic focusing, an optical interaction region to detect light scatter and fluorescence, and an interaction region to detect impedance change induced by each cell. *Id.* According to Kummrow, since only one sheath inlet is necessary, easy handling of the structures is ensured. *Id.*

Figure 1(a) of Kummrow is reproduced below:

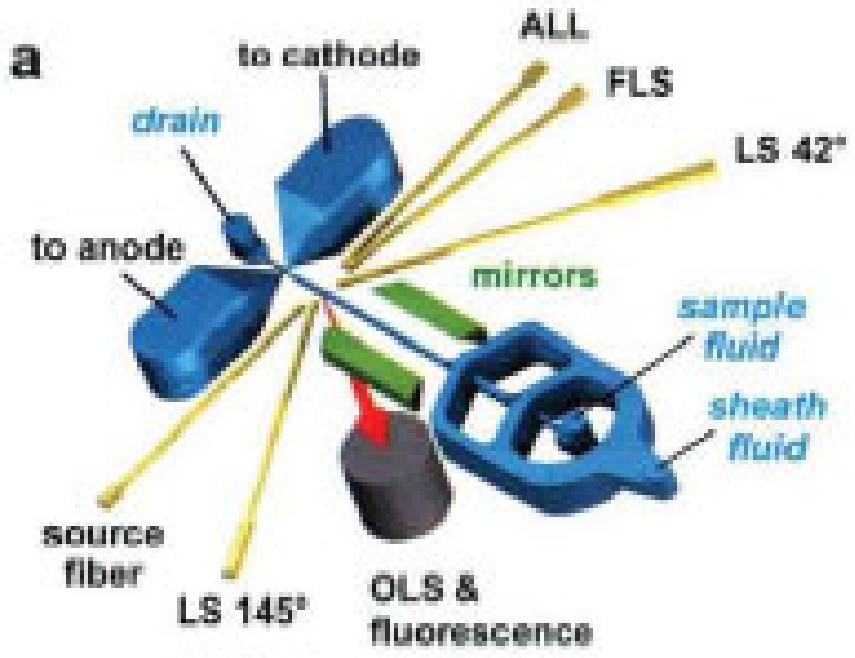


Figure 1(a) of Kummrow is a schematic diagram of a micro-fluidic flow cell featuring two stage cascaded hydrodynamic focusing, integrated mirrors (green), grooves for optical fibers (yellow), and fluidic connections (blue) to externally mounted platinum electrodes. *Id.*

2. Analysis of Claim 9

Claim 9 depends from independent claim 1 and further recites "wherein each of the fluid focusing features is in fluid communication with a first focusing fluid inlet port provided on a top surface of the substrate." Ex. 1001, 19:40–43.

Petitioner does not rely on Kummrow to remedy the deficiency in the ground of obviousness of claim 1 over Simonnet, which we have addressed above. Accordingly, we determine that Petitioner has not established that claim 9 would have been obvious over Simonnet and Kummrow.

III. CONCLUSION

We conclude that Petitioner has not established that any of the challenged claims are unpatentable.

IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that on the record before us, Petitioner has not shown by a preponderance of the evidence that claims 1, 2, 6, 8, and 9 of the '439 patent are unpatentable; and

FURTHER ORDERED that this is a Final Written Decision. Parties to the proceeding seeking judicial review of the decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

In summary:

Claims	35 U.S.C. §	Reference(s)/Basis	Claims Shown Unpatentable	Claims Not Shown Unpatentable
1, 2, 6, 8	102	Simonnet		1, 2, 6, 8
1, 2, 6, 8	103	Simonnet		1, 2, 6, 8
8	103	Simonnet, Kim		8
9	103	Simonnet, Kummrow		9
Overall Outcome				1, 2, 6, 8, 9

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(54) **HYDRODYNAMIC FOCUSING APPARATUS AND METHODS**

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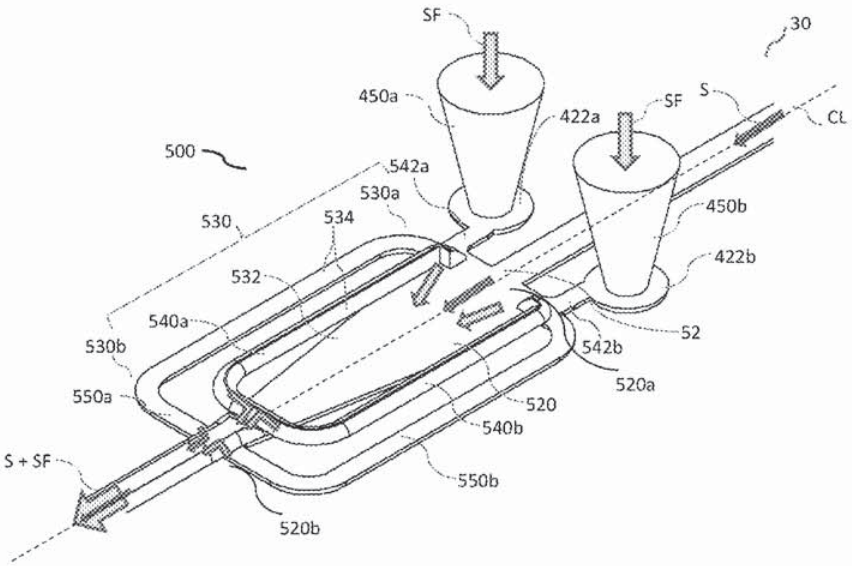
(57) **ABSTRACT**

A microfluidic chip having a micro channel for processing a sample is provided. The micro channel may focus the sample by using focusing fluid and a core stream forming geometry. The core stream forming geometry may include a lateral fluid focusing component and one or more vertical fluid focusing components. A microfluidic chip may include a plurality micro channels operating in parallel on a microfluidic chip.

23 Claims, 20 Drawing Sheets



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U.S. Appl. No. 11/998,557, filed Nov. 30, 2007, now U.S. Pat. No. 7,611,309, issued.
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U.S. Appl. No. 13/968,962, filed Aug. 16, 2013, now U.S. Pat. No. 9,446,912, issued.
U.S. Appl. No. 15/269,556, filed Sep. 19, 2016, now U.S. Pat. No. 9,802,767, issued.
U.S. Appl. No. 15/797,790, filed Oct. 30, 2017, publication No. 2018-0208412, published.
Cytonome/ST, LLC, the assignee of the instant application, is a party to the case of *Inguran, LLC d/b/a STGenetics, XY, LLC, and Cytonome/ST, LLC* v. *ABS Global, Inc., Genus PLC, and Premium*

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Cytonome/ST, LLC is the Assignee of U.S. Pat. No. 7,611,309. The '309 Patent was involved in an Inter Partes Review proceeding under case number IPR2017-02161 (Institution Denied).
Cytonome/ST, LLC is the Assignee of U.S. Pat. No. 9,446,912. The '912 Patent is involved in ongoing Inter Partes Review proceedings under case number IPR2017-02162.
Cytonome/ST, LLC is the Assignee of U.S. Pat. No. 7,311,476. The '476 Patent was involved in an Inter Partes Review proceeding under case number IPR2017-02163 (Institution Denied).
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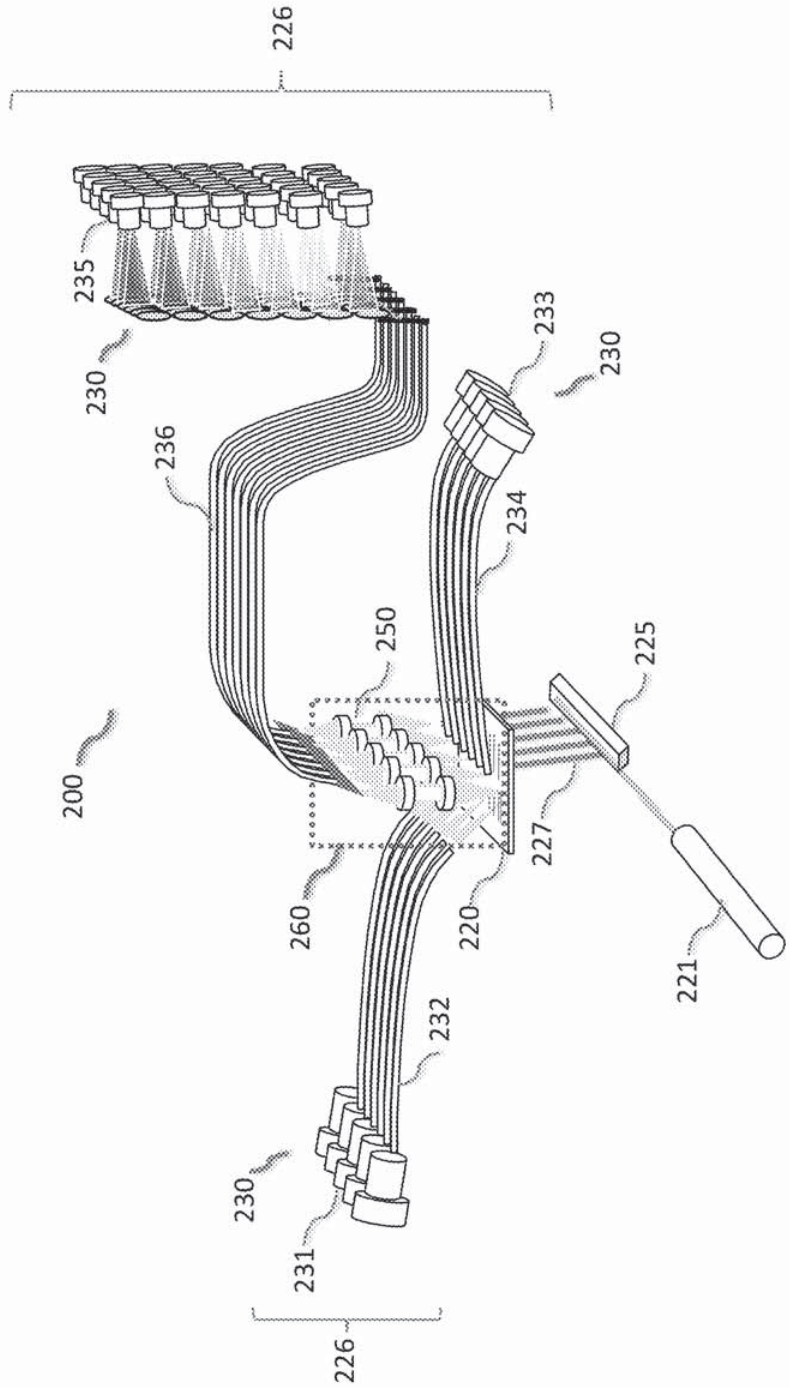
FIG. 1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226

FIG. 2

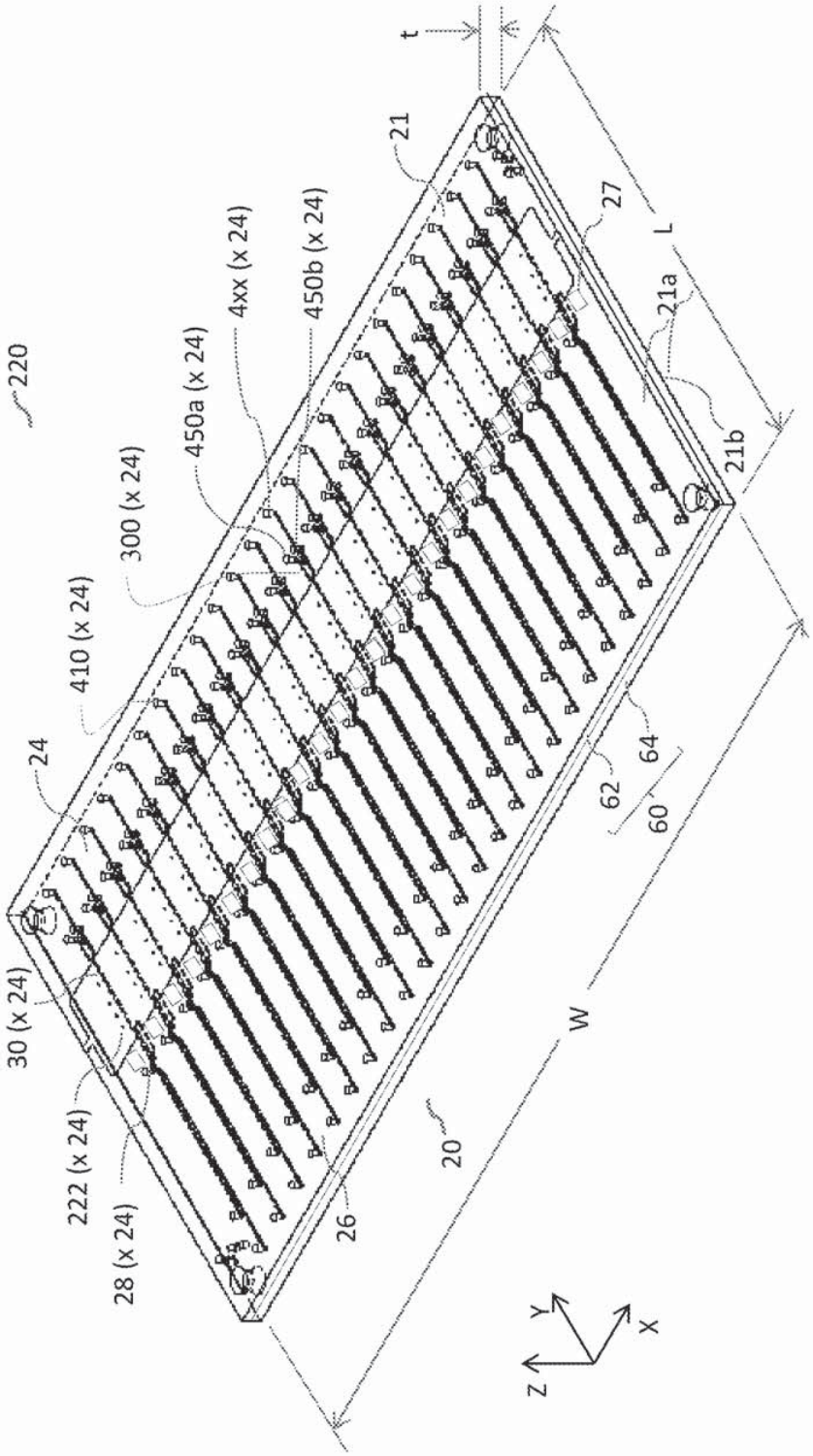
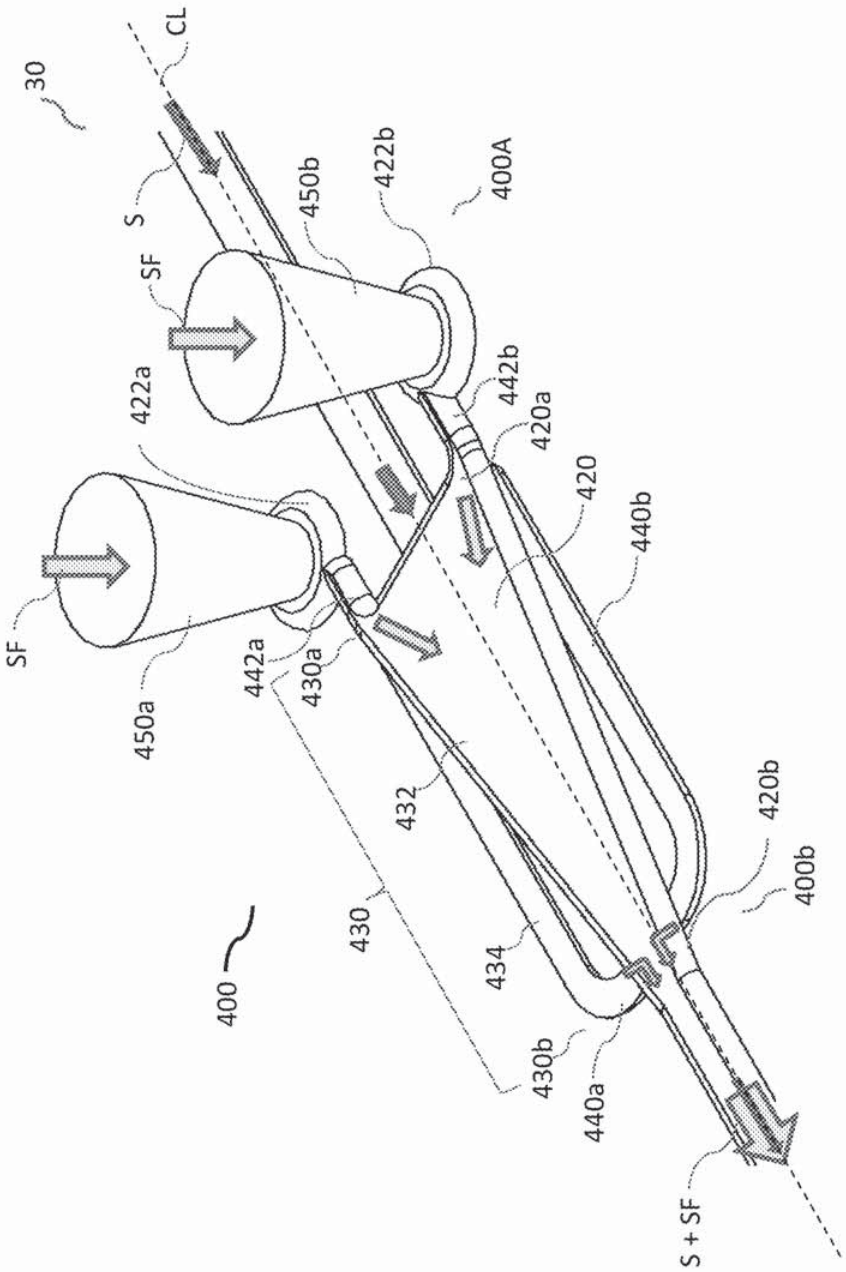

t
21
27
L
21a
21b
220
450a (x 24)
4xx (x 24)
450b (x 24)
300 (x 24)
410 (x 24)
24
62
64
60
30 (x 24)
222 (x 24)
28 (x 24)
26
20
W
Y
X
Z

FIG. 3A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CL
S
SF
450b
422b
400A
422a
442b
420a
420
440b
SF
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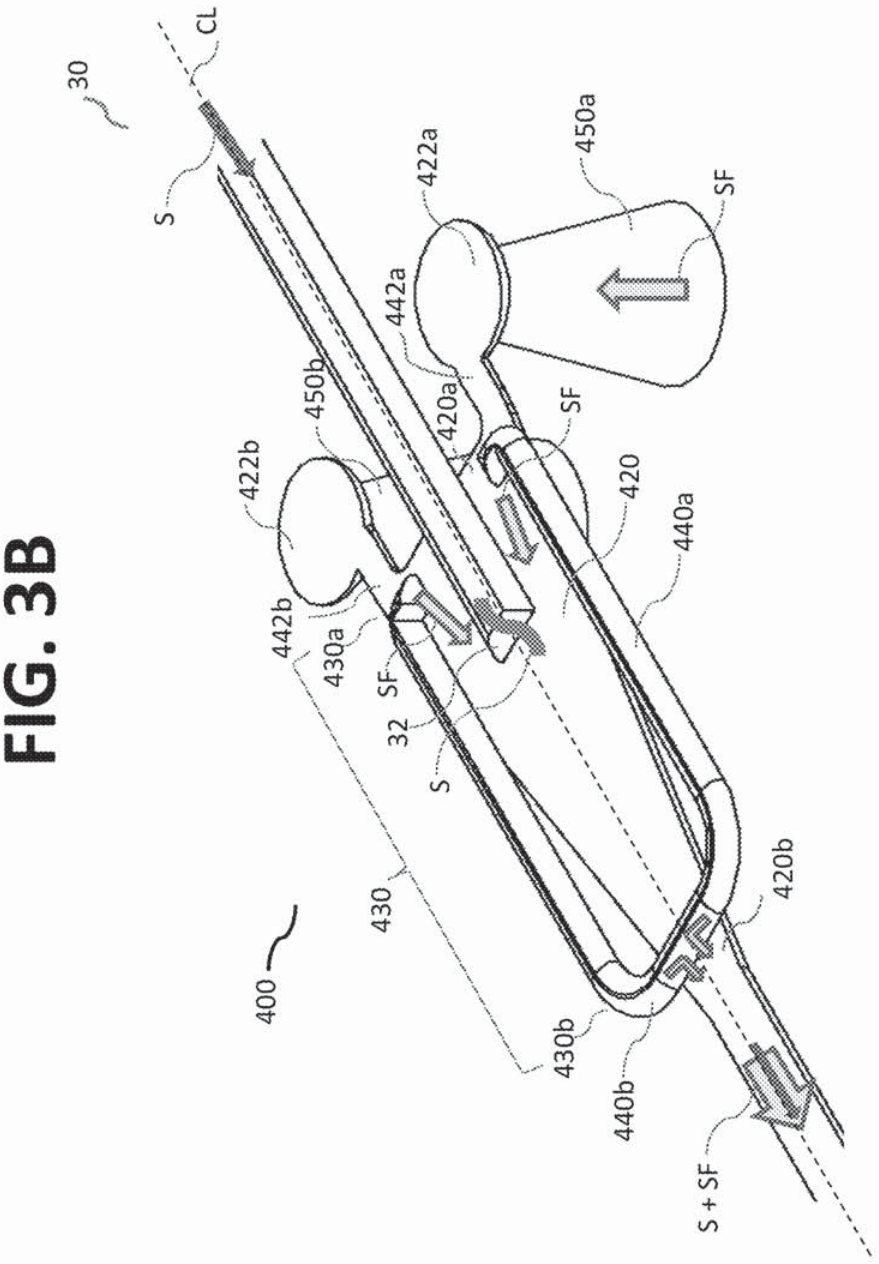
442a
430a
432
420b
400b
430
434
400
430b
440a
S + SF

FIG. 3B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CL
S
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450a
SF
442a
450b
420a
SF
422b
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SF
32
S
430
400
420b
430b
440b
S + SF

FIG. 3C

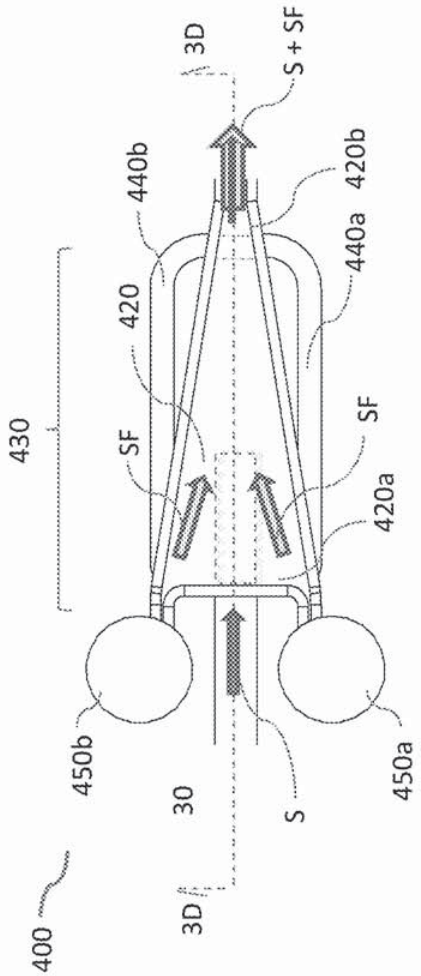
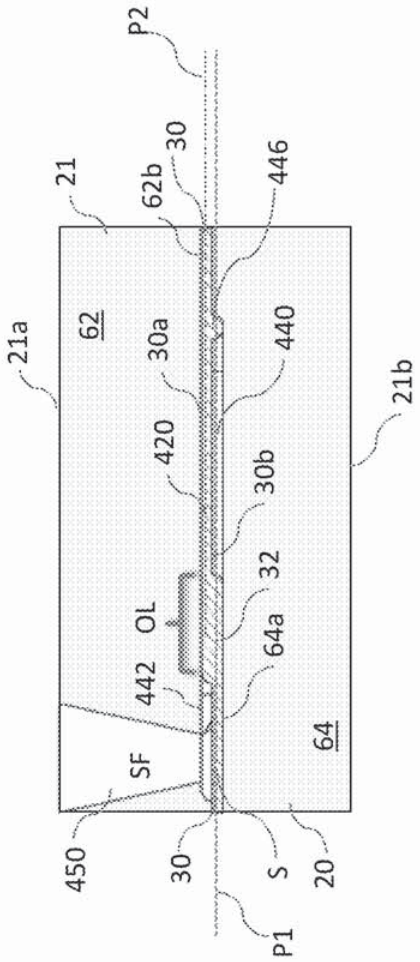


FIG. 3D


P2
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62b
30
446
21a
62
30a
440
21b
420
30b
32
OL
64a
442
64
SF
450
30
S
20
P1

FIG. 3E

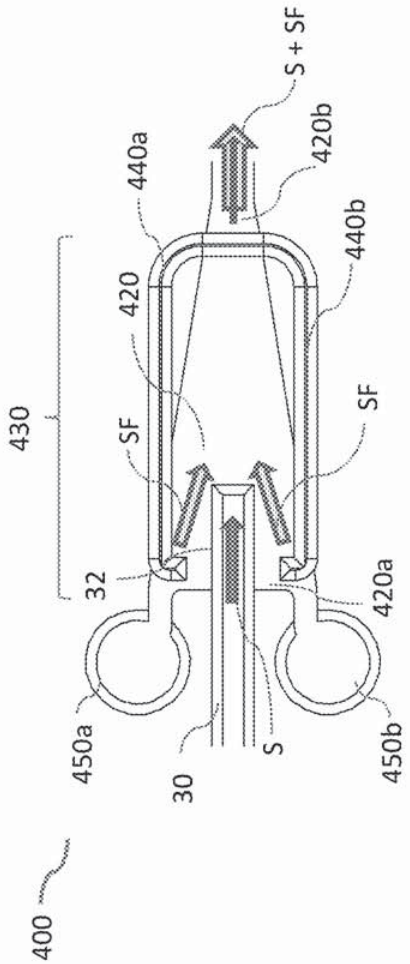
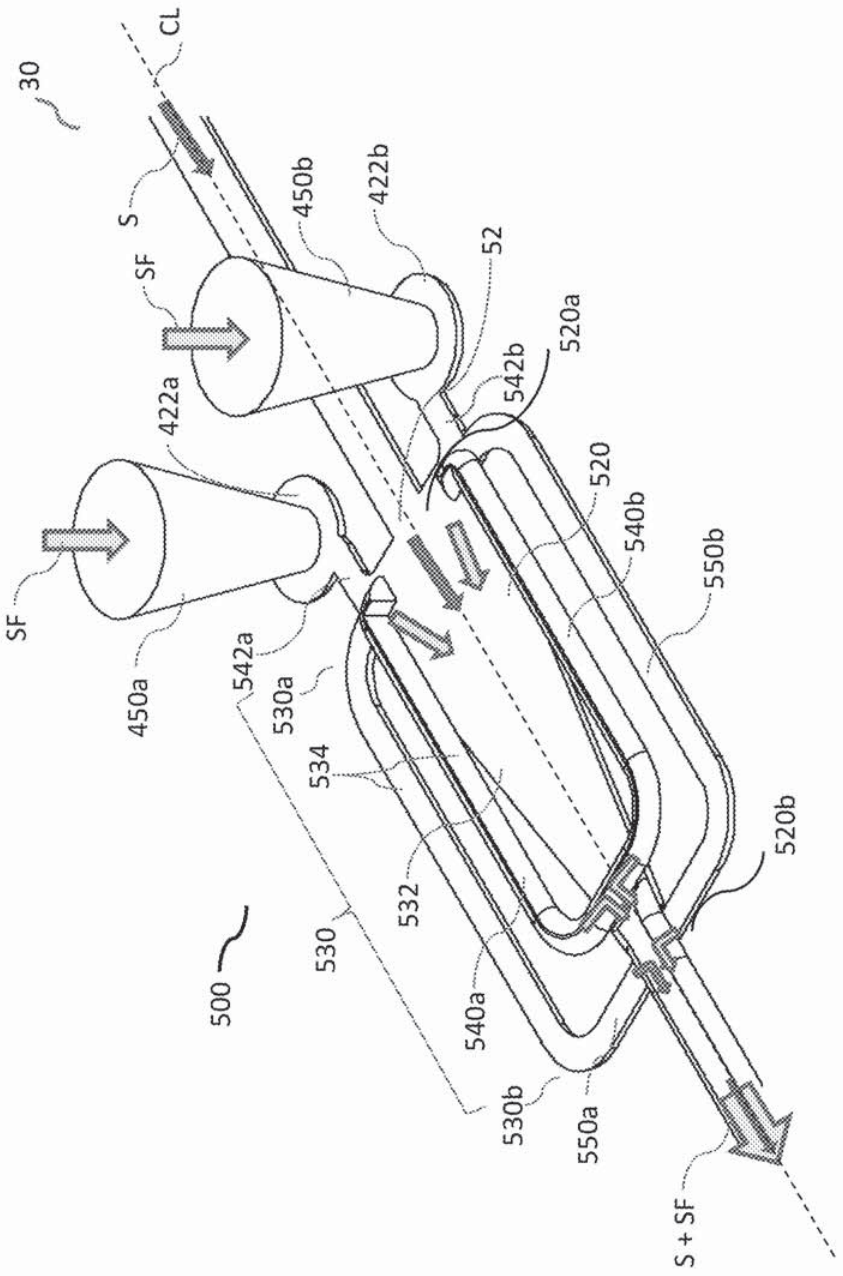


FIG. 4A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CL
S
SF
450b
422b
52
520a
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422a
520
540b
550b
SF
450a
542a
530a
534
532
520b
530
500
540a
530b
550a
S + SF

FIG. 4B

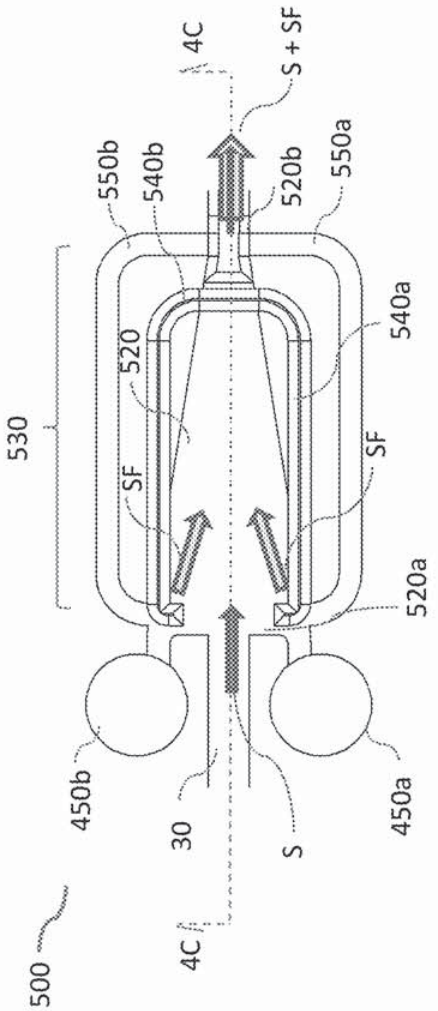
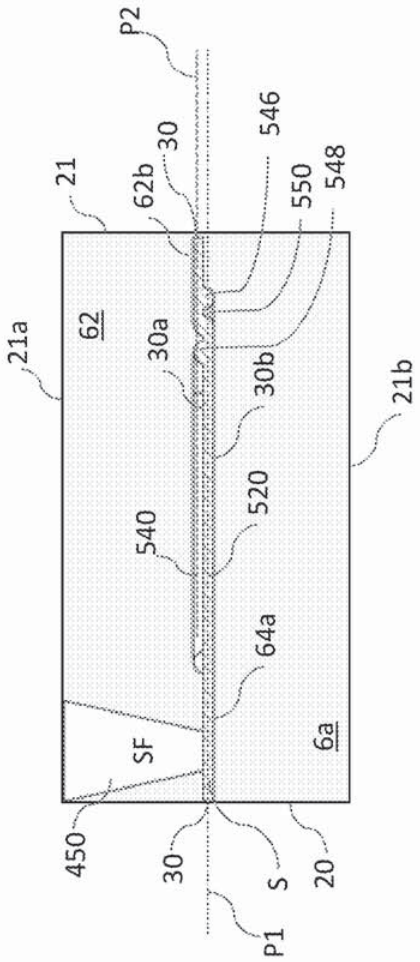

4C
S + SF
550b
540b
520b
550a
540a
520
530
SF
SF
520a
450b
30
S
450a
500
4C

FIG. 4C


P2
546
30
21
62b
550
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21a
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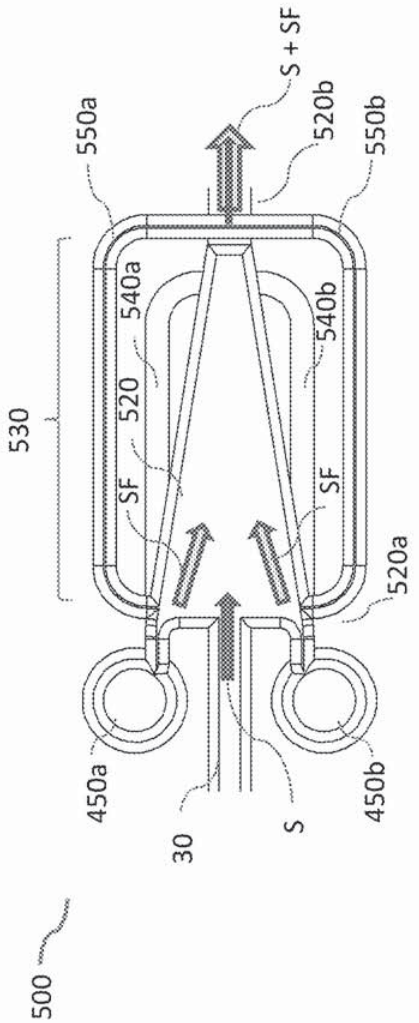
30a
30b
21b
520
540
64a
SF
6a
450
30
S
20
P1

FIG. 4D


S + SF
550a
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550b
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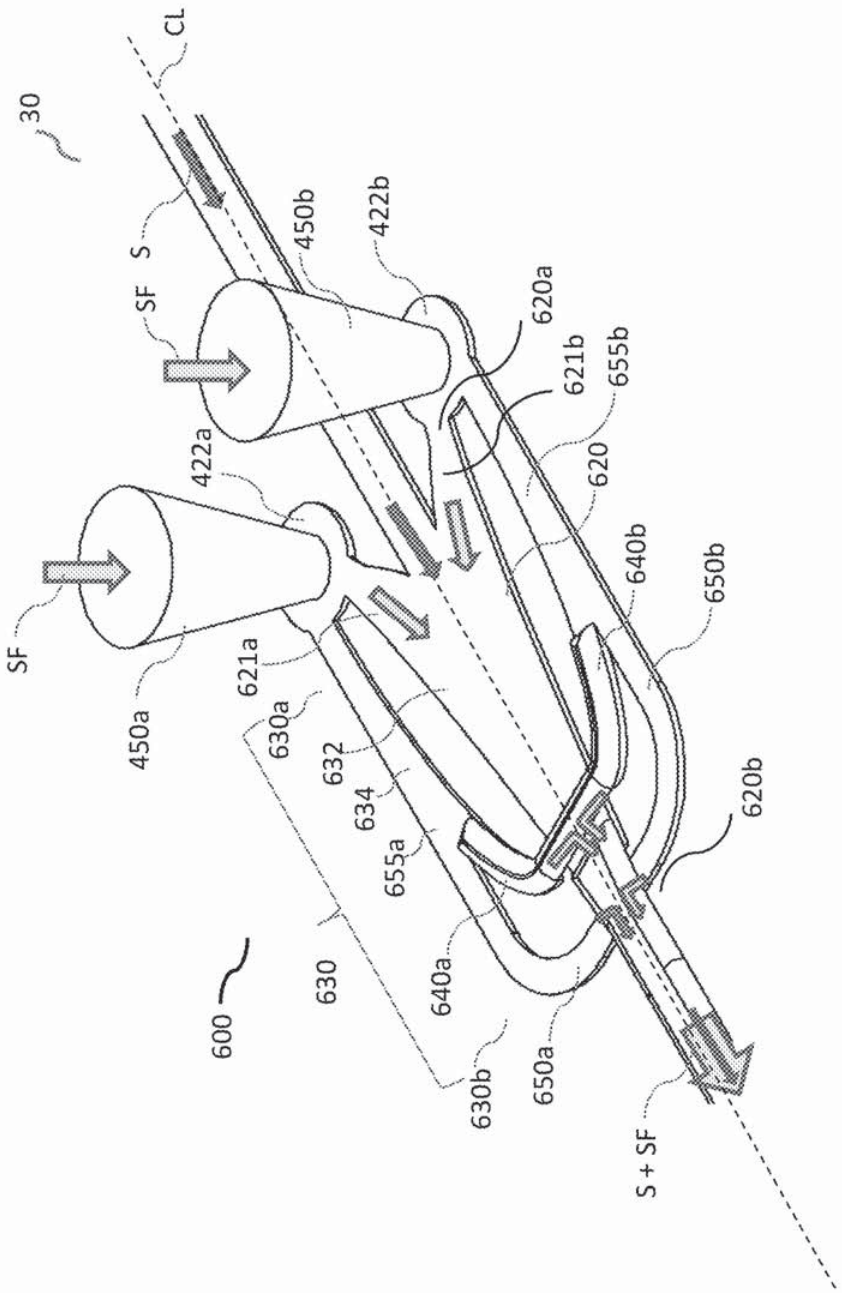
530
520
SF
SF
520a
450a
30
S
450b
500

FIG. 5A


CL
30
S
SF
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422a
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655a
620b
630
600
640a
650a
630b
S + SF

FIG. 5B

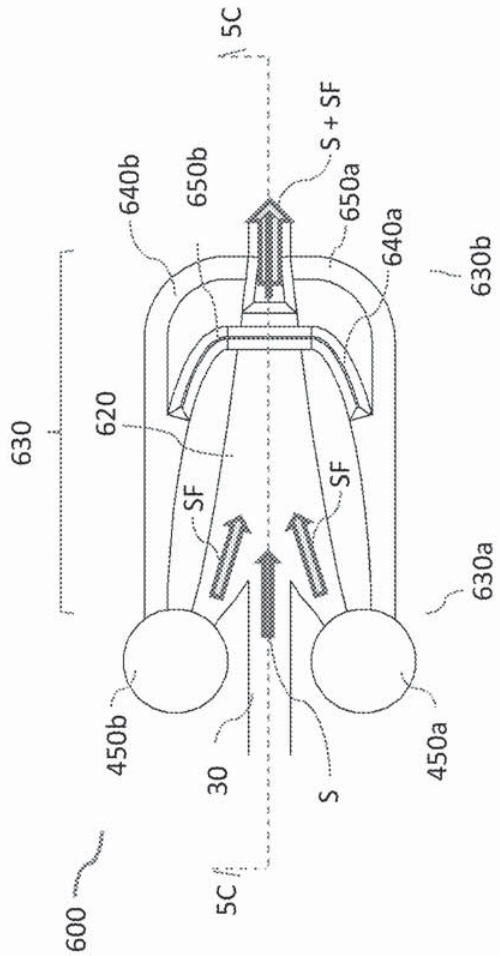
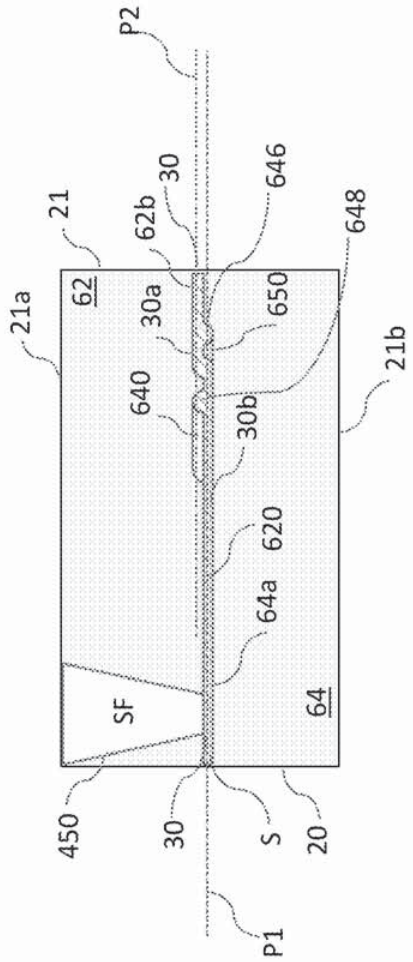

5C
S + SF
640b
650b
650a
640a
630b
620
630
SF
SF
630a
450b
30
S
450a
600
5C

FIG. 5C


P2
21
62b
30
646
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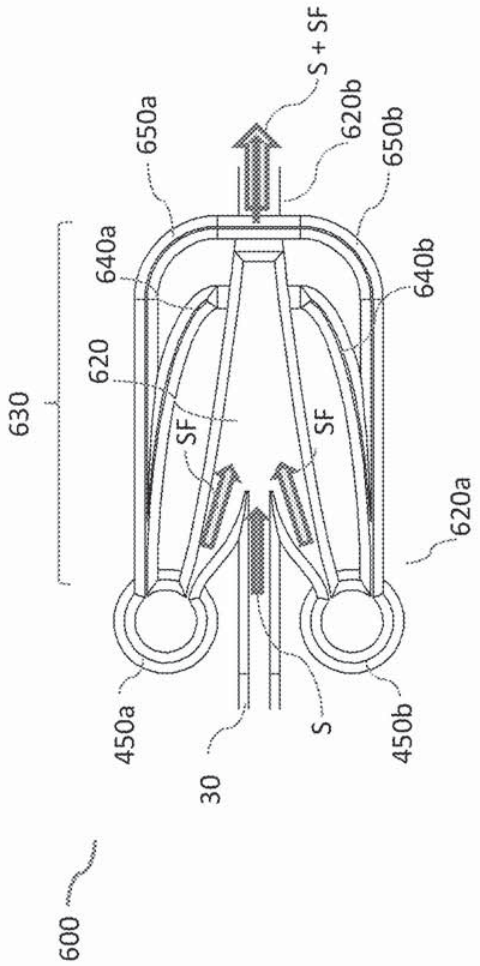
62
30a
650
21a
21b
640
30b
620
64a
SF
64
450
30
S
20
P1

FIG. 5D


S + SF
650a
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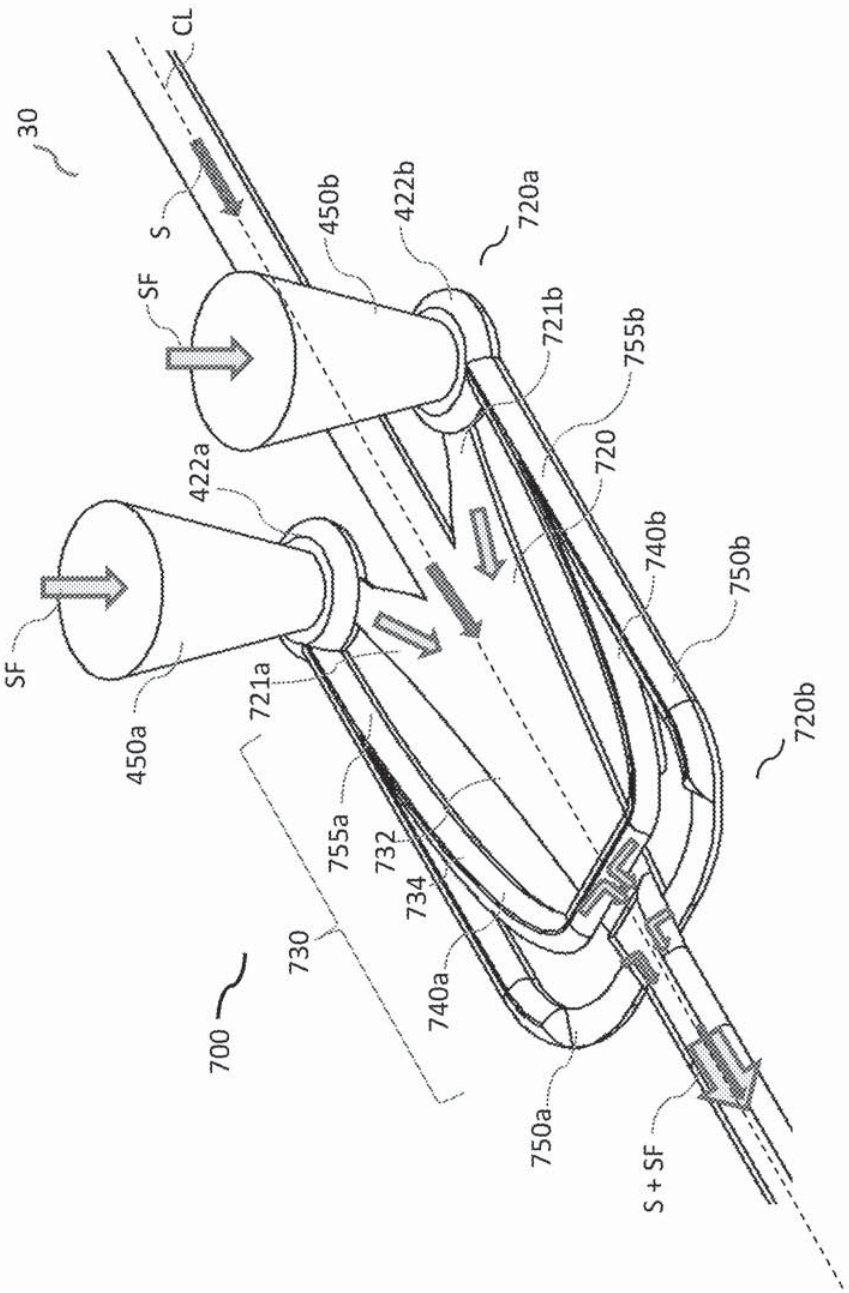
640b
620
630
SF
SF
620a
450a
S
450b
30
600

FIG. 6A


30
CL
S
SF
450b
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720a
721b
755b
720
422a
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720b
755a
732
734
730
740a
700
750a
S + SF

FIG. 6B

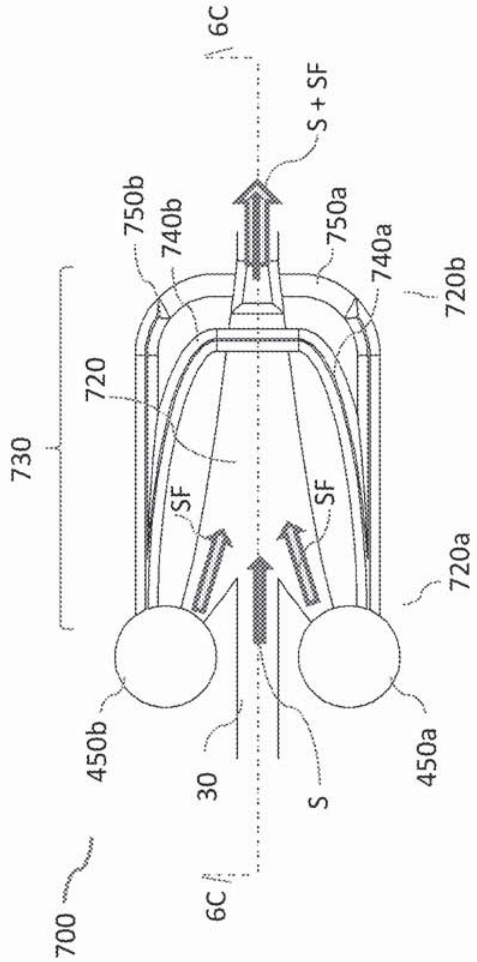
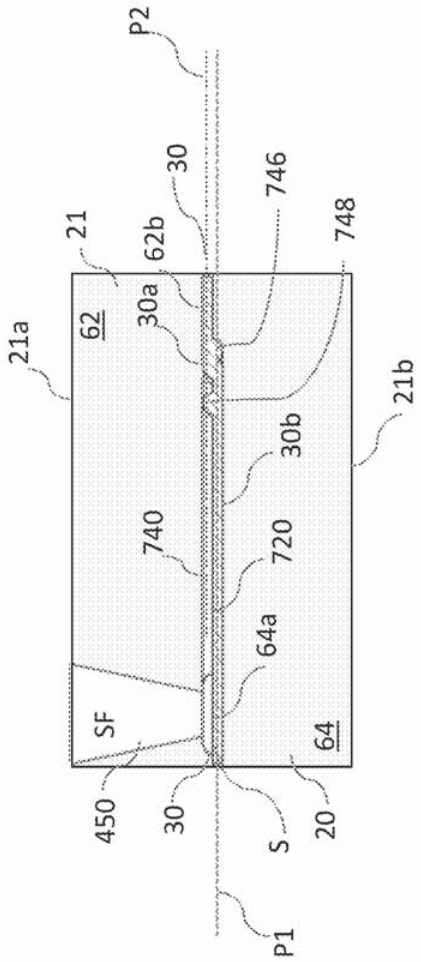

6C
S + SF
750b
740b
750a
740a
720b
720
730
SF
SF
720a
450b
30
S
450a
700
6C

FIG. 6C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SF
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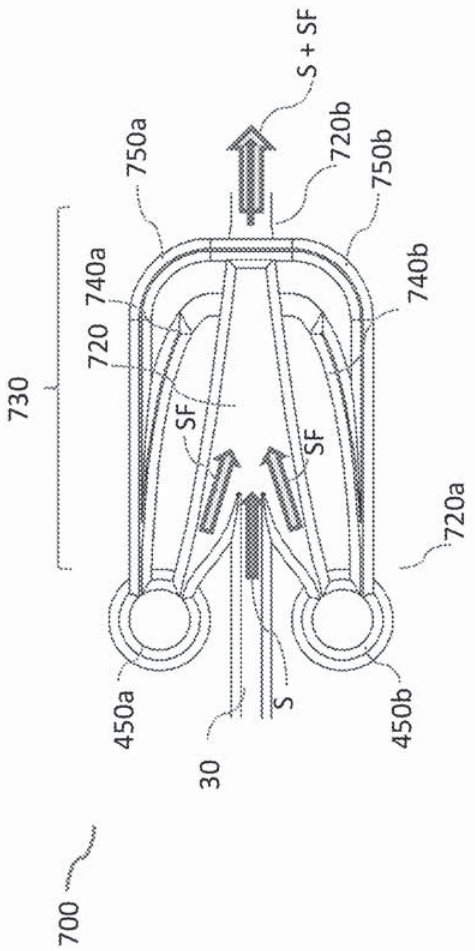
450
30
S
20
P1

FIG. 6D


S + SF
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SF
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720a
450a
S
450b
30
700

FIG. 7C

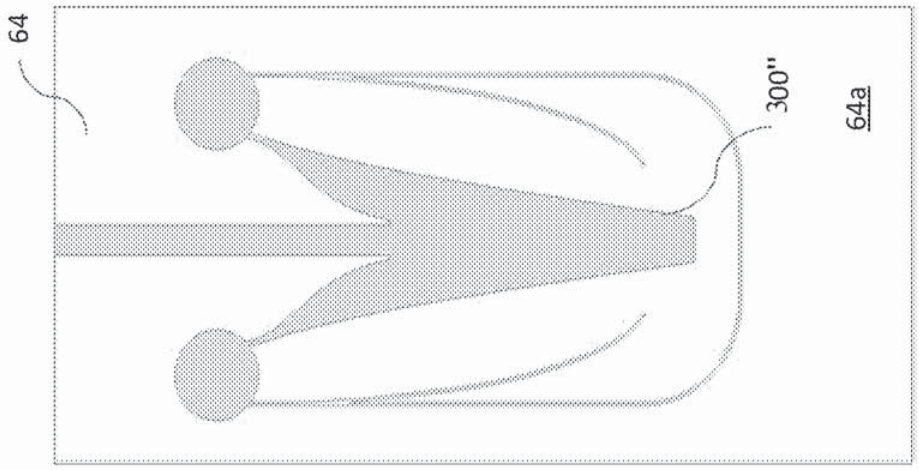


FIG. 7B

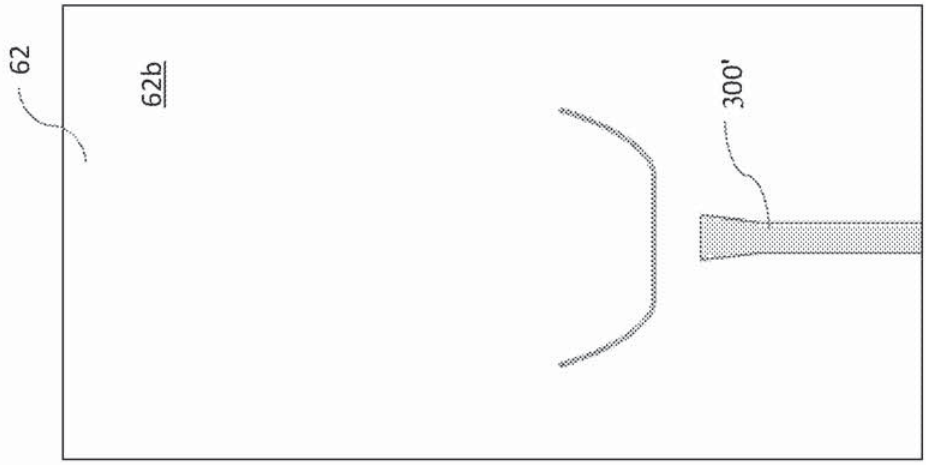
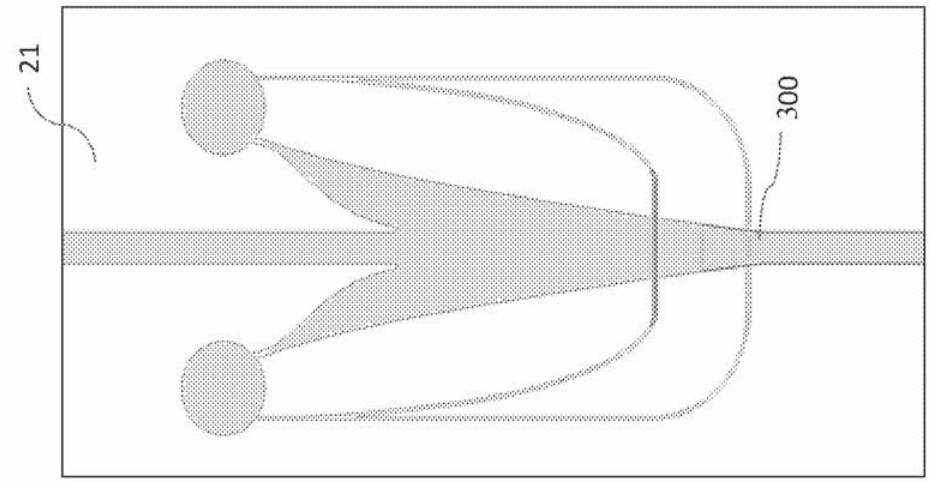


FIG. 7A



HYDRODYNAMIC FOCUSING APPARATUS AND METHODS

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application Ser. No. 61/785,734, titled "Hydrodynamic Focusing Apparatus and Methods," and filed Mar. 14, 2013, the content of which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

Generally, this disclosure relates to hydrodynamic focusing, in particular, in a microfluidic device. More specifically, the present disclosure relates to systems and methods for producing a sheath flow in a flow channel and, in particular, in a micro channel in a microfluidic device.

BACKGROUND

Sheath flow is a particular type of laminar flow in which one layer of sample fluid, or a particle, is surrounded by another layer of focusing fluid on more than one side. The process of confining a particle stream in a fluid is referred to as a 'sheath flow' configuration. For example, in a sheath flow configuration, a sheath fluid may envelop and pinch a sample fluid containing a number of particles. The flow of the sample fluid containing particles suspended therein may be narrowed almost to the outer diameter of particles in the center of the sheath fluid. The resulting sheath flow flows in a laminar state within an orifice or channel so that the particles are aligned and accurately pass through an orifice or channel in a single file row.

Sheath flow is used in many applications where it is preferable to protect particles or fluids by a layer of sheath fluid, for example in applications wherein it is necessary to protect particles from air. For example, in particle sorting systems, flow cytometers and other systems for analyzing a sample, particles to be sorted or analyzed are usually supplied to a measurement position in a central fluid current, which is surrounded by a particle free liquid sheath.

Sheath flow is useful because it can position particles with respect to sensors or other components and prevent particles in the center fluid, which is surrounded by the sheath fluid, from touching the sides of the flow channel and thereby prevents clogging of the channel. Sheath flow allows for faster flow velocities and higher throughput of sample material. Faster flow velocity is possible without shredding cells in the center fluid because the sheath fluid protects the cells from potentially high shear forces at the walls of the flow channel.

Conventional devices that have been employed to implement sheath flow have relatively complex designs and are relatively difficult to fabricate.

SUMMARY

According to aspects of the disclosure, a microfluidic particle processing assembly including a substrate and a flow channel formed in the substrate may be provided. The flow channel may include an inlet, a fluid focusing region having an associated fluid focusing feature for focusing a particle within the flow channel, and an inspection region at least partially downstream of the fluid focusing region. Further, the flow channel may have first and second outlets.

According to other aspects, the fluid focusing features of the flow channel focusing region may include a core stream forming geometry. The core stream forming geometry may further include a lateral fluid focusing region, a first vertical fluid focusing component, and a second vertical fluid focusing component.

According to some aspects, the first vertical fluid focusing component may include a vertical fluid focusing channel and the second vertical fluid focusing component may include a second vertical fluid focusing channel. The first vertical fluid focusing component and the second vertical fluid focusing component may be in communication with the fluid focusing region in opposite vertical directions. The first vertical fluid focusing component may provide a first vertical influence and the second vertical fluid focusing component may provide a second vertical influence in the opposite directions as the first vertical influence.

According to other aspects, the flow channel may further include a sheath inlet in fluid communication with the sheath source. A sample inlet may be positioned within a sheath flow created by the sheath inlet to facilitate a co-axial flow of sheath and sample. The sample inlet may include a tapered or beveled inlet.

According to yet other aspects, the flow channel may have a first width and a first height at the sample inlet. The flow channel may a second width and a second height at a first transition point. The height of the flow channel may be reduced between the sample inlet and the first transition point. The flow channel may have a third width and a third height at a second transition point. The height of the flow channel may remain constant between the first transition point and the second transition point and the width of the flow channel may be reduced between the first transition point and the second transition point. The third height and the third width of the flow channel may be maintained through the inspection region. The flow channel may transition from a square cross section to a rectangular cross section. The flow channel may transition from a circular cross section to an elliptical cross section.

The microfluidic assembly may further include a plurality of flow channels as presented herein.

According to other aspects, the fluid focusing feature of the fluid focusing region may further include ultrasonic transducers for producing pressure waves in the focusing region of each flow channel. The ultrasonic transducers may be an array of ultrasonic transducers for producing a standing pressure wave along the flow channel.

According to even other aspects, a diverting mechanism in communication with the flow channel may be provided. The diverting mechanism may include a bubble valve. Alternatively, the diverting mechanism may include an array of ultrasonic and/or standing acoustic wave transducers. Optionally, the diverting mechanism may include interdigitated transducers (IDT).

According to certain aspects, a microfluidic chip may include a substantially planar chip substrate having an upper surface and a lower surface. A microfluidic flow channel may be provided within the chip substrate. A first inlet port may be formed on the upper surface of the chip substrate for receiving a focusing fluid. The first inlet port may be in fluid communication with the microfluidic flow channel. The microfluidic flow channel may include a first focusing fluid inlet configured to introduce focusing fluid from the first inlet port into the microfluidic channel in a first direction, a second focusing fluid inlet configured to introduce focusing fluid from the first inlet port into the microfluidic channel in a second direction, and a third focusing fluid inlet configured

to introduce focusing fluid from the first inlet port into the microfluidic channel in a third direction.

According to certain other aspects, the microfluidic chip may also include a second inlet port formed on the upper surface of the chip substrate for receiving a focusing fluid. The second inlet port may be in fluid communication with the microfluidic flow channel. The microfluidic flow channel may include a fourth focusing fluid inlet configured to introduce focusing fluid from the second inlet port into the microfluidic channel in a fourth direction. The second focusing fluid inlet may be configured to introduce focusing fluid from the second inlet port into the microfluidic channel in the second direction, and the third focusing fluid inlet may be configured to introduce focusing fluid from the second inlet port into the microfluidic channel in the third direction.

The microfluidic flow channel may include a fluid flow focusing region having an upstream end region and a downstream end region. The first focusing fluid inlet may be configured to introduce focusing fluid into the fluid flow focusing region in the upstream end region. The second and third focusing fluid inlets may be configured to introduce focusing fluid into the fluid flow focusing region in the downstream end region.

According to other aspects, a microfluidic chip may include a substantially planar substrate having an upper surface and a lower surface. A microfluidic channel may be formed in the substantially planar substrate and may have an upper surface and a lower surface. An inlet port may be formed on the upper surface of the substantially planar substrate and may be configured to receive a focusing fluid. A first focusing fluid channel in fluid communication with the inlet port may be provided. The first focusing fluid channel may be configured to introduce focusing fluid into the microfluidic channel via a first aperture in the upper surface of the microfluidic channel. A second focusing fluid channel in fluid communication with the inlet port may be provided. The second focusing fluid channel may be configured to introduce focusing fluid into the microfluidic channel via a second aperture in the lower surface of the microfluidic channel.

The microfluidic channel and the first and second focusing fluid channels may be formed when a lower surface of an upper substrate layer and an upper surface of a lower substrate layer are joined together.

The microfluidic channel may lie in a first plane upstream of the first aperture and in a second plane downstream of the second aperture.

At least one outlet port may be formed on the upper surface of the substantially planar substrate and in fluid communication with the fluid flow focusing region.

Certain embodiments of the disclosed apparatus and methods are summarized below. These embodiments are not intended to limit the scope of the disclosure, but rather serve as brief descriptions of exemplary embodiments. Both the disclosure and claimed invention may encompass a variety of forms which differ from these summaries.

BRIEF DESCRIPTION OF THE DRAWINGS

Exemplary embodiments of the present disclosure are further described with reference to the appended figures. It is to be noted that the various features and combinations of features described below and illustrated in the figures can be arranged and/organized differently to result in embodiments which are still within the spirit and scope of the present disclosure. To assist those of ordinary skill in the art in making and using the disclosed systems, assemblies and methods, reference is made to the appended figures.

FIG. **1** schematically illustrates an exemplary particle processing system according to the present disclosure.

FIG. **2** illustrates an exemplary microfluidic chip according to the present disclosure;

FIG. **3**A is a top perspective view of a portion of a flow channel geometry with arrows schematically depicting flow of sample fluid and focusing fluid in accordance with certain embodiments described herein.

FIG. **3**B is a bottom perspective view of a portion of a flow channel geometry in accordance with the embodiment of FIG. **3**A, with arrows schematically depicting flow of sample fluid and focusing fluid in accordance with certain embodiments described herein.

FIG. **3**C is top view of a portion of a flow channel geometry in accordance with the embodiment of FIG. **3**A.

FIG. **3**D is a cross-section through line **3**D-**3**D of FIG. **3**C of a portion of a flow channel geometry in accordance with the embodiment of FIG. **3**A

FIG. **3**E is bottom view of a portion of a flow channel geometry in accordance with the embodiment of FIG. **3**A.

FIG. **4**A is a top perspective view of a portion of a flow channel geometry with arrows schematically depicting flow of sample fluid and focusing fluid in accordance with certain embodiments described herein.

FIG. **4**B is top view of a portion of a flow channel geometry in accordance with the embodiment of FIG. **4**A.

FIG. **4**C is a cross-section through line **4**C-**4**C of FIG. **4**B of a portion of a flow channel geometry in accordance with the embodiment of FIG. **4**A.

FIG. **4**D is bottom view of a portion of a flow channel geometry in accordance with the embodiment of FIG. **4**A.

FIG. **5**A is a top perspective view of a portion of a flow channel geometry with arrows schematically depicting flow of sample fluid and focusing fluid in accordance with certain embodiments described herein.

FIG. **5**B is top view of a portion of a flow channel geometry in accordance with the embodiment of FIG. **5**A.

FIG. **5**C is a cross-section through line **5**C-**5**C of FIG. **5**B of a portion of a flow channel geometry in accordance with the embodiment of FIG. **5**A.

FIG. **5**D is bottom view of a portion of a flow channel geometry in accordance with the embodiment of FIG. **5**A.

FIG. **6**A is a top perspective view of a portion of a flow channel geometry with arrows schematically depicting flow of sample fluid and focusing fluid in accordance with certain embodiments described herein.

FIG. **6**B is top view of a portion of a flow channel geometry in accordance with the embodiment of FIG. **6**A.

FIG. **6**C is a cross-section through line **6**C-**6**C of FIG. **6**B of a portion of a flow channel geometry in accordance with the embodiment of FIG. **6**A.

FIG. **6**D is bottom view of a portion of a flow channel geometry in accordance with the embodiment of FIG. **6**A.

FIG. **7**A is top view of a portion of a substrate of the microfluidic chip, schematically illustrating micro channel geometry, in accordance with the embodiment of FIG. **5**A, formed between an upper substrate layer and a lower substrate layer.

FIG. **7**B is bottom view of a portion of an upper substrate layer in accordance with the embodiment of FIGS. **5**A and **7**A, schematically illustrating the micro channel geometry formed in the lower surface of the upper substrate layer.

FIG. **7**C is top view of a portion of a lower substrate layer in accordance with the embodiment of FIGS. **5**A and **7**A,

schematically illustrating the micro channel geometry formed in the upper surface of the lower substrate layer.

While the present disclosure may be embodied with various modifications and alternative forms, specific embodiments are illustrated in the figures and described herein by way of illustrative examples. It should be understood the figures and detailed descriptions are not intended to limit the scope of the claims to the particular form disclosed, but that all modifications, alternatives, and equivalents falling within the spirit and scope of the claims are intended to be covered.

DETAILED DESCRIPTION

A microfluidic particle (e.g., cell) analysis and/or sorting system for a microfluidic chip, in accordance some embodiments, may have a wide variety of applications as a therapeutic medical device enabling cell-based therapies, such as blood transfusion, bone marrow transplants, and/or mobilized peripheral blood implants. Embodiments of microfluidic sorting systems may be capable of selecting cells based on intrinsic characteristics as determined by interaction of light with the cells (e.g., scatter, reflection, and/or auto fluorescence) independent of protocols and necessary reagents. A microfluidic system may employ a closed, sterile, disposable cartridge including a microfluidic chip. The microfluidic system may process particles (e.g., cells) at high speeds, and deliver particles (e.g., cells) with high yield and high purity.

Certain embodiments described herein relate systems and methods for producing a sheath flow in a flow channel and, in particular, in a micro channel in microfluidic devices.

As used herein, the term "particles" includes, but is not limited to, cells (e.g., blood platelets, white blood cells, tumorous cells, embryonic cells, spermatozoa, etc.), synthetic beads (e.g., polystyrene), organelles, and multi-cellular organisms. Particles may include liposomes, proteoliposomes, yeast, bacteria, viruses, pollens, algae, or the like. Particles may also refer to non-biological particles. For example, particles may include metals, minerals, polymeric substances, glasses, ceramics, composites, or the like. Additionally, particles may include cells, genetic material, RNA, DNA, fragments, proteins, etc. or bead, for example, with fluorochrome conjugated antibodies.

As used herein, the term "microfluidic system" refers to a system or device including at least one fluidic channel having microscale dimensions. The microfluidic system may be configured to handle, process, detect, analyze, eject, and/or sort a fluid sample and/or particles within a fluid sample. The term "channel" as used herein refers to a pathway formed in or through a medium that allows for movement of fluids, such as liquids and gases. The term "micro channel" refers to a channel, preferably formed in a microfluidic system or device, having cross-sectional dimensions in the range between about 1.0 μm and about 2000 μm, preferably between about 25 μm and about 500 μm, and most preferably between about 50 μm and about 300 μm. One of ordinary skill in the art will be able to determine an appropriate volume and length of the micro channel for the desired application. The ranges above are intended to include the above-recited values as upper or lower limits. The micro channel may have any selected cross-sectional shape or arrangement, non-limiting examples of which include a linear or non-linear configuration, a U-shaped or D-shaped configuration, and/or a rectangular, triangular, elliptical/oval, circular, square, or trapezoidal geometry. A microfluidic device or microfluidic chip may include any suitable number of micro channels for transporting fluids. A microfluidic chip may be provided as a disposable cartridge with a closed channel system.

As used herein the terms "vertical," "lateral," "top," "bottom," "above", "below," "up," "down," and other similar phrases should be understood as descriptive terms providing general relationship between depicted features in the figures and not limiting on the claims, especially relating to flow channels and microfluidic chips described herein, which may be operated in any orientation.

The present disclosure bears relations to U.S. Pat. No. 7,311,476 which is hereby incorporated by reference.

Referring now to FIG. **1**, a particle processing system **200** may be configured, dimensioned and adapted for analyzing, sorting, and/or processing (e.g., purifying, measuring, isolating, detecting, monitoring and/or enriching) particles (e.g., cells, microscopic particles, etc.) or the like. For example, system **200** may be a cytometer and/or a cell purification system or the like, although the present disclosure is not limited thereto. Rather, system **200** may take a variety of forms, and it is noted that the systems and methods described may be applied to other particle processing systems.

In exemplary embodiments, system **200** is a microfluidic flow sorter particle processing system (e.g., microfluidic chip based system) or the like. Exemplary microfluidic flow sorter particle processing systems and components or the like are disclosed, for example, in U.S. Pat. No. 8,529,161 (Ser. No. 13/179,084); U.S. Pat. No. 8,277,764 (Ser. No. 11/295,183); U.S. Pat. No. 8,123,044 (Ser. No. 11/800,469); U.S. Pat. No. 7,569,788 (Ser. No. 11/101,038); U.S. Pat. No. 7,492,522 (Ser. No. 11/906,621) and U.S. Pat. No. 6,808,075 (Ser. No. 10/179,488); and US Patent Publication Nos. 2012/0277902 (Ser. No. 13/342,756); 2011/0196637 (Ser. No. 13/022,525) and 2009/0116005 (Ser. No. 12/259,235); and U.S. Patent Application Ser. Nos. 61/647,821 (Ser. No. 13/896,213) and 61/702,114 (Ser. No. 14/029,485), 61/784,323, the foregoing being incorporated herein by reference in their entireties.

In further exemplary embodiments, system **200** may be a multi-channel or multi-jet flow sorter particle processing system (e.g., multiple capillaries or multiple fluid jet-based systems) or the like. Exemplary multi-channel or multi-jet flow sorter particle processing systems and components or the like are disclosed, for example, in US Patent Publication No. 2005/0112541 (Ser. No. 10/812,351), the entire contents of which is hereby incorporated by reference in its entirety.

FIG. **1** illustrates a system **200** suitable for implementing an illustrative embodiment of the present disclosure. System **200** includes a microfluidic assembly **220**. Microfluidic assembly **220** includes and/or is in communication with a particle inspection region and a sample fluid input region. Microfluidic assembly **220** may include a plurality of micro channels for conveying a substance, such as particles or cells, therethrough. In certain embodiments and as can be understood by those familiar in the art, microfluidic assembly **220** may be a combination of microfluidic chips, micro channels, cuvettes, capillaries, nozzles, or jets which may combine to produce a multichannel particle processing system. The micro channels transport fluid and/or particles through the assembly **220** for processing, handling, and/or performing any suitable operation (e.g., on a liquid sample). Assembly **220** may include any suitable number of micro channels for transporting fluids through assembly **220**.

In exemplary embodiments, an optical detector system **226** for use with microfluidic assembly **220** may be provided. Optical detector system **226** may be configured for

the interrogation of the particles flowing through or located within an interrogation region. Further, optical detector system **226** may monitor flow through a plurality of channels simultaneously. In exemplary embodiments, system **226** can inspect individual particles for one or more particular characteristics, such as size, form, fluorescence, optical scattering, as well as other characteristics.

System **200** also includes at least one electromagnetic radiation or light source **221** (e.g., a laser source or the like) for simultaneously or sequentially illuminating at least a portion of each of an interrogation region. The electromagnetic radiation source **221** may be coupled to and/or in communication with beam shaping optics **225** (e.g., segmented mirror/mirrors or the like) for producing and forming a beam of electromagnetic radiation (e.g., light) **227**. The light source **221** may be provide as one or more monochromatic light sources, polychromatic light sources, or any combination of the aforementioned. In general, the electromagnetic radiation source(s) **221** may have any suitable wavelength and one skilled in the art will recognize that any suitable light source(s) may be used.

In some embodiments, the one or more radiation beams **227** may pass through an optical mask aligned with a plurality of particle-conveying micro channels in the microfluidic assembly **220**. The optical mask may take the form of an array of pinholes (e.g., provided in an optically opaque layer) associated with the interrogation regions of the plurality of micro channels. Other spatial and/or spectral filter arrays may be provided in the illumination and/or detection path of the particle processing system **200**.

Examples of optical signals that may be produced in optical particle analysis, cytometry and/or sorting when a beam **227** intersects a particle include, without limitation, optical extinction, angle dependent optical scatter (forward and/or side scatter) and fluorescence. Optical extinction refers to the amount of electromagnetic radiation or light that a particle extinguishes, absorbs, or blocks. Angle dependent optical scatter refers to the fraction of electromagnetic radiation that is scattered or bent at each angle away from or toward the incident electromagnetic radiation beam. Fluorescent electromagnetic radiation may be electromagnetic radiation that is absorbed and/or scattered by molecules associated with a particle or cell and re-emitted at a different wavelength. In some instances, fluorescent detection may be performed using intrinsically fluorescent molecules.

In exemplary embodiments, optical detector system **226** may include one or more detector subsystems **230** to capture and observe the optical signals generated by the intersection of electromagnetic radiation beam **227** with a particle in a channel. Detector subsystems **230** may include one or more extinction detector assemblies **231** for capturing extinction signals, one or more scatter detector assemblies **233** for capturing scatter signals, and one or more fluorescence detector assemblies **235** for capturing fluorescence signals. In a preferred embodiment, detector system **226** may include at least one extinction detector assembly **231**, at least one scatter detector assembly **233**, and at least one fluorescence detector assembly **235**. Detector assemblies **231**, **233**, **235** may include photomultipliers, photodiodes, cameras, or other suitable device(s).

According to certain aspects, a detector subsystem **230** may include one or more micro-lens systems **250**. A plurality of micro-lens systems **250** may be provided as a micro-lens array **260**. Further, detector subsystems **230** may include fiber optics or other waveguide-type optical transmission elements to direct the signals to the sensor elements, one or more lenses, filters, mirrors, and/or other optical elements to collect, shape, transmit, etc. the signal exiting the interrogation region **222** and being received by the detector subsystems **230**.

According to certain embodiments, a single detector subsystem **230** may be associated with a plurality of interrogation sites (e.g., microfluidic channels) and thus, may receive signals (simultaneously, sequentially, overlapping, non-overlapping, etc.) from each of the plurality of interrogation sites. The detector subsystems **230** may be connected to electronics (not shown) to analyze the signals received from the detector assemblies and/or control one or more aspects of the particle sorting system **200**.

According to certain embodiments and referring to FIG. **2**, microfluidic assembly **220** may be configured as a microfluidic chip **20** and may include a substrate **21** having a plurality of channels **30** (e.g., micro channels) disposed or formed therein. The micro channels **30** may be configured to transport fluid and/or particles through the microfluidic chip **20** for processing, handling, and/or performing any suitable operation on a liquid sample (e.g., a particle sorting system). For example, each micro channel **30** may be a flow cytometer. According to certain aspects, the micro channels **30** may be arranged parallel to each other.

As best shown in FIG. **2**, the microfluidic chip **20** may include an input region **24** in which a sample containing particles (e.g., cells, etc.) is input into the microfluidic chip **20** for processing and an output region **26** for removing the processed sample from the microfluidic chip **20**. The substrate **21** may be provided as a substantially planar substrate, i.e., having a first dimension (e.g., thickness t) much less than its other two dimensions (e.g., length L and width W). Further, the substrate **21** of the microfluidic chip **20** may include first and second major plane surfaces: an upper surface **21***a*; and a lower surface **21***b*.

The sample fluid may be input via a sample inlet port **410** through the upper surface **21***a* of the microfluidic chip **20**. Each micro channel **30** may have an interrogation region **222** associated therewith. Particles in channels **30** may be detected while flowing through the interrogation regions **222**. At the interrogation region **222**, individual particles may be inspected or measured for a particular characteristic, such as size, form, orientation, fluorescence intensity, etc. Interrogation regions **222** may be illuminated through the upper surface **21***a* and/or the lower surface **21***b* of the microfluidic chip **20**.

The plurality of channels **30** may be evenly distributed (i.e., evenly spaced) across the width W of the microfluidic chip **20**. According to certain embodiments, a centerline-to-centerline spacing between the channels **30** may range from 0.2 mm to 5.0 mm. The centerline-to-centerline spacing between the micro channels **30** may be less than 4.0 mm, less than 3.0 mm, or even less than 1.0 mm. According to certain embodiments, the centerline-to-centerline spacing between the micro channels **30** may range from 2.5 mm to 3.0 mm. Advantageously, to minimize the footprint of the microfluidic chip **20**, the centerline-to-centerline spacing between the micro channels **30** may be less than 2.0 mm, less than 1.5 mm, or even less than 1.0 mm. According to certain embodiments, the centerline-to-centerline spacing between the micro channels **30** may range from 0.7 mm to 1.2 mm.

The substrate **21** of the microfluidic chip **20** may be formed with one or more substrate layers **60**. As shown in FIG. **2**, the substrate **21** may be formed by bonding or otherwise attaching an upper substrate layer **62** to a lower substrate layer **64**. In general, any number of layers may be used to form microfluidic chip **20**.

The substrate layers **60** of the microfluidic chip **20** may be glass (e.g., UV fused-silica, quartz, borofloat, etc.), PDMS, PMMA, COC, or any other suitably transmissive material. The thickness of the first substrate layer **62** may range from approximately 100 μm up to approximately 1000 μm. In certain preferred embodiments, the thickness of substrate layer **62** may range from approximately 200 μm up to approximately 600 μm. For example, the thickness of substrate layer **62** may be approximately 400 μm. In other preferred embodiments, the thickness of substrate layer **62** may range from approximately 500 μm up to approximately 900 μm. By way of non-limiting examples, the thickness of substrate layer **62** may be approximately 700 μm or approximately 750 μm. In certain embodiments, the microfluidic chip **20** may be formed with only two substrate layers **62**, **64**.

In the embodiment illustrated in FIG. **2**, the microfluidic chip **20** includes twenty-four micro channels **30**, although, in general, any number of micro channels **30** may be provided (e.g., as non-limiting examples, 2, 4, 8, 24, 36, 72, 144, or 288 channels). According to some embodiments, when microfluidic chip **20** has twenty-four micro channels **30**, the microfluidic chip **20** may have an overall width W ranging from 70 mm to 80 mm.

According to certain embodiments, each of the plurality of micro channels **30** may include a sorting or diverting mechanism **28** for directing particles flowing within the channels **30** into various downstream channels. Sorting and/or diverting may be accomplished through one or more mechanisms, which may include but are not limited to: mechanical displacement of the particle by deflecting a membrane with a piezoelectric actuator, thermal actuators, optical force techniques, dielectric methods (e.g., dielectrophoretic), ultrasonic transducers **27** (both bulk and/or surface), surface acoustic wave actuators, and other suitable sort mechanisms or techniques. A surface acoustic wave actuator may be provided as an interdigitated transducer (IDT). Exemplary ultrasonic transducers are disclosed, for example, in U.S. patent Ser. Nos. 12/631,059 and 13/818,146, the entire contents of which are hereby incorporated by reference in their entirety.

The particle processing system **200** may include a receptacle or holder (not shown) for removably receiving microfluidic chip **20**. Further, the particle processing system **200** may include one or more stages for positioning the microfluidic chip **20** relative to the optical detection system **226**. The stages may allow for movement (translation and/or rotation) of the microfluidic chip **20**.

According to aspects of the disclosure, a microfluidic chip having a micro channel for processing a sample fluid is provided. The micro channel **30** may be in fluid communication with one or more sample inlet ports **410** (see FIG. **2**) configured to receive a sample fluid S. The sample inlet ports **410** may be in fluid communication with a sample reservoir, manifold, channel, well, test tube, etc. Further, the micro channel **30** may be in fluid communication with one or more focusing fluid inlet ports **450** (e.g., **450***a* and **450***b*) configured to receive a focusing fluid SF. The focusing fluid inlet ports **450** may be in fluid communication with a sheath fluid reservoir, manifold, channel, bag, bottle, container, etc.

According to aspects of the disclosure, the micro channel **30** may focus the sample by using focusing fluid (e.g., sheath fluid) and a core stream forming geometry **300**, for example, defined in the substrate **21** of the microfluidic chip **20**. The core stream forming geometry **300** may be used to laminarly focus, streamline, decelerate, and/or accelerate the flow of the sample fluid S with a surrounding sheath of focusing fluid SF. In some embodiments, the core stream forming geometry **300** may include a lateral fluid focusing component (see, for example, lateral fluid focusing component **432** of the embodiment of FIGS. **3**A-E) and one or more vertical fluid focusing components (see, for example, vertical fluid focusing component **434** of FIGS. **3**A-E). In the context of the embodiment of FIG. **2**, "lateral" may refer to a direction extending generally in the plane of the substantially planar microfluidic chip **20** and "vertical" may refer to a direction extending generally out of the plane of the microfluidic chip **20**.

Referring now to FIGS. **3**A and **3**B, a portion of a micro channel **30** having a core stream forming geometry **400** is shown. A sample fluid S flowing through the micro channel **30** may enter the core stream forming geometry **400** along a longitudinal centerline CL (when viewed from above) of the core stream forming geometry **400**. Focusing fluid SF may enter the core stream forming geometry **400** symmetrically with respect to the longitudinal centerline CL of the core stream forming geometry **400**. The focusing fluid may enter the core stream forming geometry **400** at an upstream region **400***a* of the core stream forming geometry and also at a downstream region of the core stream forming geometry **400***b*. The sample fluid S and the focusing fluid SF may be induced to flow through the micro channel **30** via any means known in the art, including one or more pumps (e.g., peristaltic pumps), ultrasonic drivers, etc.

The core stream forming geometry **400** may include a fluid focusing region **430** incorporated into a region of a flow channel **30** for generating a focused core stream flow wherein the focusing fluid SF shapes the sample stream S. The core stream forming geometry **400** is illustrated as interior surfaces of a flow channel **30** in a microfluidic chip **20**, such as those microfluidic chips previously described. The illustrated core stream forming geometry **400** provides improved sheath flow capabilities and thus improved sample focusing capabilities. The core stream forming geometry **400** may be fabricated in plastics, polycarbonate, glass, metals, or other suitable materials using microfabrication, injection molding, stamping, machining, 3D printing or by other suitable fabrication techniques. As such, the core stream forming geometry **400** may be formed in a single substrate layer, or by a plurality of stacked layers.

Referring to FIGS. **3**A and **3**B, sheath inlets ports **450** may be provided with conical inlet shapes that are each received at a sheath aggregating volume **422**. The sheath aggregating volumes **422** may be provided with a single outlet or sheath fluid channel **442**, or multiple outlets or sheath fluid channels to further transport focusing fluid SF to flow channel **30** components. In some embodiments, there may not be any feature specifically identifiable as a sheath aggregating volume and focusing fluid may flow directly from sheath inlet ports **450** to a focusing fluid distribution network.

In FIGS. **3**A and **3**B, two sheath inlet ports **450***a*, **450***b* are associated with a single micro channel **30**. Each sheath inlet port **450** may provided with a single port outlet or sheath fluid channel **440**. Sheath fluid channel **440***a* is illustrated as extending from sheath fluid inlet port **450***a* and sheath fluid channel **440***b* is illustrated as extending from sheath fluid inlet port **450***b*. Each sheath fluid channel **440** extends from an upstream region **430***a* of the fluid focusing region **430** to a downstream region **430***b* of the fluid focusing region **430**. Each sheath fluid channel **440** is configured to transport focusing fluid SF from a sheath inlet port **450** to the micro channel **20** in the downstream region **430***b* of the fluid focusing region **430**. In the embodiment of FIGS. **3**A-**3**E, the core stream forming geometry **400** is symmetrically formed

relative to a longitudinal centerline CL of the micro channel **30** (when viewed from above).

According to alternative embodiments, a single sheath fluid inlet port **450** may be provided and a branched sheath fluid channel may be configured transport focusing fluid form the single sheath fluid inlet port **450** to a plurality of regions of the core stream forming geometry **400**. Additionally, flow restrictions may be placed on one or more fluidic paths emanating from the sheath aggregating volume **422**.

The fluid focusing region **430** may include a lateral fluid focusing component **432** and a vertical fluid focusing component **434**, both of which may contribute to shaping the sample stream S and increasing the axial acceleration of both the focusing or sheath fluid FS and sample S through the flow channel **30**. The lateral fluid focusing component may include a lateral fluid focusing chamber **420**. The lateral fluid focusing chamber **420** is provided with sample fluid S from a portion of the micro channel **30** in fluid communication with the sample inlet port **410**. Further, the lateral fluid focusing chamber **420** is provided with sheath or focusing fluid SF from the one or more sheath fluid inlet ports **450**.

According to the embodiment of FIGS. **3**A-**3**E, the lateral fluid focusing chamber **420** is widest at its upstream end **420***a* and narrowest at its downstream end **420***b*. Between the upstream end **420***a* and the downstream end **420***b*, the chamber **420** substantially linearly tapers symmetrically with respect to the centerline CL in the lateral direction. Between the upstream end **420***a* and the downstream end **420***b*, the chamber **420** has a substantially constant thickness. Further, the upstream end **420***a* is provided as a substantially flat wall having two openings, one at each corner, for admitting focusing fluid SF.

Thus, as illustrated, two sheath inlet ports **450***a*, **450***b* may symmetrically introduce focusing fluid SF into the lateral fluid focusing chamber **420**. In FIGS. **3**A-**3**E, a relatively short channel **442***a* extends between the sheath aggregating volume **422***a* and the corner opening of the lateral fluid focusing chamber **420**. Similarly, a relatively short channel **442***b* extends between the sheath aggregating volume **422***b* and laterally opposed corner opening of the lateral fluid focusing chamber **420**. Thus, focusing fluid SF enters chamber **420** from opposed lateral edges (or lateral sides) of the upstream end **420***a* of focusing chamber **420**.

As best shown in FIGS. **3**B, **3**D and **3**E, at the upstream end **420***a* of focusing chamber **420**, a sample inlet portion **32** of the micro channel **30** transporting sample fluid S extends beneath the plane of the lateral fluid focusing chamber **420**. The sample inlet portion **32** of micro channel **30** is centered along the longitudinal centerline CL. The sample S is injected into the plane of the focusing chamber **42** through the opening where the sample inlet portion **32** of the micro channel **30** and the lateral fluid focusing chamber **420** overlap OL. As shown in FIG. **3**E, the length of the overlap OL is approximately a third of the length of the focusing chamber **420**. In other words, the sample inlet portion **32** and the lateral fluid focusing chamber **420** share a common opening (where otherwise they would have shared a common wall). Sample fluid S enters focusing chamber **420** from below via a symmetrically centered opening having a length equal to the overlap OL region and a width equal to the width of micro channel **30**. Thus, in this embodiment, the sample stream S jogs from the plane of the upstream micro channel **30** upward into the focusing fluid SF within the plane of the focusing chamber as it is introduced into the focusing chamber.

As the sample stream and the focusing fluid progress along the lateral fluid focusing chamber **420** the lateral dimension of the chamber **420** decreases. As the chamber **420** narrows or tapers in the lateral direction as the fluid travels downstream, an increasing inward force from the lateral sides of the chamber **420** acts on the fluid within the chamber, thus tending to focus (e.g., constrict) the sample S in the middle of the lateral fluid focusing chamber **420**. The increasing inward force further tends to accelerate both the sheath and the sample within the fluid focusing region **430** in the flow channel **30**.

At the downstream end **420***b* of the lateral fluid focusing chamber **420**, the vertical fluid focusing component provides a vertical upwardly-directed focusing force. Specifically, vertical fluid focusing channels **440***a*, **440***b* introduce focusing fluid FS from inlet ports **450***a*, **450***b* into the lateral fluid focusing chamber **420** at the downstream end **420***b*. As best shown in FIGS. **3**B, **3**D and **3**E, the vertical fluid focusing channels **440***a*, **440***b* extend under channel **30**. Where the top surface of channel **440***a* intersects the lower surface **30***b* of channel **30** an opening or aperture forms a vertical focusing flow inlet **446** so that focusing fluid FS from channels **440***a*, **440***b* may enter channel **30**. Thus, the vertical fluid focusing channels **440***a*, **440***b* introduce focusing fluid FS into fluid focusing chamber **420** at vertical focusing flow inlet **446** from below.

Referring now to FIG. **3**A, **3**B, **3**C or **3**E, the vertical fluid focusing channels **440***a*, **440***b* may comprise a U-shaped or looping channel that branches away from the lateral fluid focusing chamber **420** and is provided in fluid communication at aperture region **446** with the lateral fluid focusing chamber **420** further downstream. In this manner, the vertical fluid focusing channels **440** may provide a means for diverting a portion of sheath fluid that may then be reintroduced into the flow channel **30** at a later point to focus the vertical position of the core stream of sample S.

As best shown in FIGS. **3**D and **3**E, the sample S enters the fluid focusing region **430** at the upstream end **430***a* in a plane P1 (see FIG. **3**D) below the plane P2 (see FIG. **3**D) in which the lateral fluid focusing chamber **420** is located. The sample S is directed upward from plane P1 into the plane P2 of the lateral focusing chamber **420** in the overlapped region OL. Then, at the downstream end **430***b* of the fluid focusing region **430**, the laterally focused sample within a sheath of focusing fluid (S+SF) is vertically focused upward by the introduction of focusing fluid SF at the vertical focusing flow inlet **446** from below. The focused stream exits the fluid focusing region **430** in the P2 (see FIG. **3**D) plane.

FIG. **3**C is a top view of the core stream forming geometry **400**, including fluid focusing region **430** and lateral fluid focusing component **420**. A sample flow S is illustrated entering the lateral focusing chamber **420** from the micro channel **30**. Focusing fluid flow SF is illustrated entering the lateral fluid focusing chamber **420** from each sheath inlet port **450** at the upstream region **420***b* of the lateral fluid focusing chamber **420**. Further, the focusing fluid SF is introduced into the lateral fluid focusing chamber **420** from a lateral edge. In this particular embodiment, the focusing fluid SF is introduced into the lateral fluid focusing chamber **420** at a lateral, upstream corner of the fluid focusing chamber **420**.

The width of the lateral fluid focusing chamber **420** decreases in a downstream direction. In this particular embodiment, the width decreases linearly over a majority of the fluid focusing region **430**. The sheath flow SF provides an increasing shearing force on the sample S, both accelerating the flow of the sample S, spacing out particles in the

sample, and laterally focusing the sample flow into the center of the lateral fluid focusing chamber **420**.

The vertical flow of the sample S is influenced by two features of the core stream forming geometry **400**, which can be best seen in FIG. **3**D. FIG. **3**D represents a vertical cross-section along a longitudinal axis of the core stream forming geometry **400**. A first downwards vertical influence on the sample stream is created upon entry into the lateral fluid focusing chamber **420**, because the sample is introduced from under the lateral fluid focusing region **420**, so that its upward flow will be resisted by the sheath flow SF above it.

A sample flow S enters the core stream forming geometry region via micro channel **30** and via sample inlet portion **32**. The sample S reaches the end of the overlapped sample inlet region OL and moves upwards against a sheath flow SF in the plane of the lateral fluid focusing chamber **420**. Once the core stream of sample S reaches vertical focusing flow inlet **446**, the vertical fluid focusing channels **440***a*, **440***b* introduce focusing fluid SF upward, thereby directing the sample S upwards and focusing the sample S away from the bottom of the flow channel **30**.

FIG. **3**D demonstrates two notably advantageous concepts. First, the representative sample flow S reflects a non-perpendicular injection point of the sample S, e.g., via the sample inlet portion **32**. Thus, in exemplary embodiments, the sample inlet portion **32** of the micro channel **30** may be configured to introduce the sample S in substantially a same flow direction (longitudinally) as the focusing fluid SF. Second, in order to provide enhanced core formation and centering, multiple sheath fluid inlets for introducing focusing fluid SF into fluid focusing region **430** may be provided. For example, in a downstream focusing region **420***b*, vertical fluid focusing channels **440***a*, **440***b* may introduce focusing fluid SF at a vertical focusing flow inlet **446**.

The core stream forming geometry **400** accelerates and focuses the sample S and the sheath fluid SF around the centrally introduced sample S. Preferably, the fluid focusing region **430** focuses the sample S away from the sides of the micro channel. The vertically focusing component, joining the micro channel **30** downstream of the fluid focusing region **430**, provides additional focusing of the sample S within the focusing fluid SF. In the embodiment of FIG. **3**A-**3**E, this secondary focusing focuses the sample in a vertical direction from below the sample S. The combination of the lateral focusing and the vertical focusing provides three-dimensional focusing of the sheath fluid around the sample. Advantageously, the resulting flow is hydrodynamically focused on all sides of the sample S away from the walls of the flow channel **30**, with the sample S being suspended as a focused core in the approximate center of the channel **30**.

After being focused in the focusing region **430**, the sample may continue through an inspection region and a particle diverting and/or sorting region. Further, the particles may be aligned and/or oriented according to specific features in the following description and a sort action may be performed according to various mechanisms.

FIGS. **4**A-**4**D, **5**A-**5**D and **6**A-**6**D introduce various embodiments which include additional focusing regions, e.g., tertiary focusing regions, downstream of the secondary focusing regions.

Turning to FIGS. **4**A-**4**D, an alternative core stream forming geometry **500** is illustrated which incorporates a fluid focusing region **530**. Fluid focusing region **530** includes a vertical fluid focusing component **534** configured as a double horseshoe or double loop an including first and second sets of vertical fluid focusing channels **540**, **550**. This embodiment relates to a core stream forming geometry **500** having a first pair of vertical fluid focusing channel **540***a*, **540***b* and second pair of vertical fluid focusing channel **550***a*, **550***b* configured to introduce opposing vertical fluid focusing sheath flows into lateral fluid focusing chamber **520** for an improved core stream formation. Specifically, as best shown in FIGS. **4**A and **4**C, the first pair of vertical fluid focusing channel **540***a*, **540***b* introduces focusing fluid SF into the downstream end **520***b* of fluid focusing chamber **520** at vertical focusing flow inlet **548** (see FIG. **4**C) from above. The second pair of vertical fluid focusing channel **550***a*, **550***b* introduces focusing fluid SF into the downstream end **520***b* of fluid focusing chamber **520** at vertical focusing flow inlet **546** (see FIG. **4**C) from below. Vertical focusing flow inlet **548** is located upstream of vertical focusing flow inlet **546**. Thus, after being laterally focused, the stream is vertically focused downward and then vertically focused upward.

FIGS. **4**A and **4**C show that a sample inlet **52** (see FIG. **4**A) of the micro channel **30** is positioned at the same vertical plane as the lateral fluid focusing chamber **520**. Further, the lateral fluid focusing chamber **520**, the vertical fluid focusing channels **550***a*, **550***b*, and the sample inlet **52** all lie in the same plane, plane P1 (see FIG. **4**C). Additionally, vertical fluid focusing channels **540***a*, **540***b* lie in a plane P2 (see FIG. **4**C) above plane P1 (see FIG. **4**C). After being subjected to the laterally-directed focusing features of the lateral focusing chamber **520**, the vertical focusing channels **540***a*, **540***b* and the vertical focusing channels **550***a*, **550***b* introduce opposing vertical focusing forces (via vertical focusing flow inlets **548**, **546**, respectively) that act on the sample S. The focused stream exits the fluid focusing region **530** in the P2 plane. Advantageously, a more focused and/or aligned sample core stream may result.

Referring to FIG. **4**A, fluid focusing region **530** includes a lateral fluid focusing component **532** which includes lateral fluid focusing chamber **520**. Similar to the embodiment of FIGS. **3**A-**3**E, the lateral fluid focusing chamber **520** is widest at its upstream end **520***a* and narrowest at its downstream end **520***b*. Between the upstream end **520***a* and the downstream end **520***b*, the chamber **520** substantially linearly tapers symmetrically with respect to the centerline CL in the lateral direction. Between the upstream end **520***a* and the downstream end **520***b*, the chamber **520** has a substantially constant thickness. Further, the upstream end **520***a* is provided as a substantially flat wall having two openings, one at each corner, for admitting focusing fluid SF.

Thus, as illustrated, two sheath inlet ports **450***a*, **450***b* may symmetrically introduce focusing fluid SF into the lateral fluid focusing chamber **520**. Referring to FIG. **4**A, a relatively short channel **542***a* extends between the sheath aggregating volume **422***a* and the corner opening of the lateral fluid focusing chamber **520**. Similarly, a relatively short channel **542***b* extends between the sheath aggregating volume **422***b* and laterally opposed corner opening of the lateral fluid focusing chamber **520**. Thus, focusing fluid SF enters chamber **520** from opposed lateral edges (or lateral sides) of the upstream end **520***a* of focusing chamber **520**.

In contrast to the embodiment of FIGS. **3**A-**3**E and as best shown in FIG. **4**C, at the upstream end **520***a* of focusing chamber **520**, sample fluid S directly flows into the chamber **520**, in the same plane P1 in which the chamber **520** is located, via a sample inlet **52** (see FIG. **4**A) of the micro channel **30**.

Referring to FIG. **4**B, focusing fluid SF flows into lateral fluid focusing chamber **520** from sheath inlet ports **450**. The

focusing fluid SF from each inlet port **450** may be divided into three sheath flow portions. A first focusing fluid portion may enter the lateral fluid focusing chamber **520** at its upstream corners. In response to the narrowing lateral width of the lateral fluid focusing chamber **520**, the focusing fluid SF tends to focus the sample S in the center of the lateral fluid focusing channel **520**. A second focusing fluid portion from each inlet port **450** may be diverted through a vertical fluid focusing channel **550***a* (or **550***b*) and a third focusing fluid portion may be directed through a vertical fluid focusing channel **540***a* (or **540***b*).

In this embodiment, the sheath aggregating volume **522** may advantageously provide a greater cross sectional area than the end of the conical sheath inlet **450**, thus providing a beneficial volume for distributing focusing fluid at relatively high sheath flow rates through each of the sheath flow portions. Further, the length the vertical focusing channels **540***a*, **540***b* is less than the length of vertical focusing channels **550***a*, **550***b*. The shorter length of vertical focusing channels **540***a*, **540***b* means that these channels have less resistance to flow of the focusing fluid therethrough (as compared to the vertical focusing channels **550***a*, **550***b*). Thus, the volume of focusing fluid that may be introduced into the fluid focusing region **530** at vertical focusing flow inlet **548** may be greater than the volume of focusing fluid that may be introduced into the fluid focusing region **530** at vertical focusing flow inlet **546**. The relative lengths of the vertical focusing channels **540**, **550** may be modified in order control the vertical focusing of the stream. In particular a difference in the focusing fluid flow through the first set of vertical focusing channels **540** and the second set of vertical focusing channels **550** may provide for an improved ability to focus the vertical position of a core stream in a flow channel **30**. In general, it may be desirable to maintain a balance between the vertical focusing forces at the vertical focusing flow inlet **548** and the vertical focusing flow inlet **546**.

Turning now to FIG. **4**C, a vertical cross-section along a longitudinal axis of the core stream forming geometry **500** illustrates a core stream of sample S and a focusing fluid SF introduced into the flow channel **30** at substantially the same vertical position. Focusing fluid SF from the first set of vertical fluid focusing channels **540** provides a downward focusing influence on the core stream of sample S, followed by an upward focusing influence from sheath fluid provided from the second set of vertical fluid focusing channels **550**. The portion of the flow channel **30** following the opposing vertical sheath flows is at an elevated vertical position relative to the lateral fluid focusing chamber **520** and the sample inlet **52**. The portion of the flow channel **30** following the focusing region may be further manipulated in a region designed to impart orientation to particles in the core stream of sample.

FIGS. **5**A-**5**D illustrate an alternative embodiment of the core stream forming geometry **600** having substantially the same vertical cross section as the embodiment of FIGS. **4**A-**4**D (compare FIG. **4**C with FIG. **4**C). There may be certain efficiencies gained in several stream lined aspects relating to the sheath fluid flow paths illustrated in FIGS. **5**A-**5**D. In one aspect sheath fluid passes through from each sheath aggregating volume **422** into a tapered focused inlet **632** which immediately puts the focusing fluid into a trajectory for laterally focusing the core stream of sample fluid S. The tapered inlets **621***a*, **621***b* may eliminate any fluid dead zone which may be caused by blunt entry geometries.

Further, the tapered inlets **621** advantageously are configured to allow the focusing fluid SF to travel in an expanding inlet channel that so that the focusing fluid is travelling substantially parallel (or at a slight angle) to the sample fluid S flowing in the micro channel **30** immediately prior to the tapered inlets **621** merging with the channel **30**. This angle may be less than 45 degrees from the longitudinal axis of the micro channel **30**. In preferred embodiments, this angle may be less than 30 degrees, less than 25 degrees, and even less than 20 degrees. The inlets **621** may expand to the point of merger with the micro channel **30**. The configuration of the inlets **621** provides a focusing fluid flow trajectory that may be substantially aligned with the sample fluid flow. Notably, enabling the focusing fluid SF to expand and travel substantially parallel to the sample S prior to merging allows a laminar flow region to be established where all of the fluid is travelling in parallel as the fluids are merged. This streamlined merging may provide a substantial reduction in fluid mixing and turbulence at the point of merger.

Further, the tapered inlets **621** allow the lateral fluid focusing component **632** and the vertical fluid focusing component **634** to be somewhat isolated from each other. In particular, the upstream end of the vertical fluid focusing component is upstream of where sample S enters the fluid focusing chamber **620**, thus mitigating any potential for sample S to inadvertently flow in the vertical fluid focusing component **634**.

In this particular embodiment, the lateral fluid focusing chamber **620** has slightly convexly curved lateral edges.

Each of the first set of vertical fluid focusing channels **640** and the second vertical fluid focusing channels **650** are also streamlined with a common inlet **655**. However, in contrast to the embodiment of FIGS. **4**A-**4**D, in this embodiment, the cross-sectional areas of the vertical focusing channels **650**, **640** need not be constant along their length, but may vary from one portion to another. Further, the cross-sectional area of vertical fluid focusing channels **640** may be larger than the flatter cross-section area of vertical fluid focusing channels **650**. This larger cross-sectional area of vertical fluid focusing channels **640** relative to the flatter cross section of vertical fluid focusing channels **650** may allow a greater flow of vertical focusing fluid to enter chamber **620** at vertical focusing flow inlet **648** (see FIG. **5**C) than at vertical focusing flow inlet **646** (see FIG. **5**C).

The greater cross-sectional area and the shorter length of vertical focusing channels **640***a*, **640***b* mean that these channels have less resistance to flow of the focusing fluid therethrough (as compared to the vertical focusing channels **650***a*, **650***b*). Thus, the volume of focusing fluid that may be introduced into the fluid focusing region **630** at vertical focusing flow inlet **648** may be greater than the volume of focusing fluid that may be introduced into the fluid focusing region **630** at vertical focusing flow inlet **646**. The relative cross-sectional areas and/or the relative lengths of the vertical focusing channels **640**, **650** may be modified in order control the vertical focusing of the stream. In some aspects, it may be desirable to maintain a balance between the vertical focusing forces at the vertical focusing flow inlet **548** and the vertical focusing flow inlet **546**. Thus, providing varying lengths, cross-sectional areas and/or non-constant cross-sectional areas for the different vertical fluid focusing channels may allow the vertical focusing forces to be balanced.

Thus, advantageously, aspects disclosed herein allow the designer to tailor the focusing flows acting on the stream so as to optimize the position and/or shape of the focused stream within the channel.

FIGS. **6**A-**6**D illustrate another embodiment of the core stream forming geometry **700**. Similar to the embodiment of

FIGS. **5**A-**5**D and as best shown in FIG. **6**A, this embodiment also has streamlined fluid focusing flow components, such as a dedicated tapered inlet **721** extending into the lateral fluid focusing chamber **720** from the inlet port **450** and a common focusing fluid channel **755** connected directly to the sheath aggregating volume **422** of each sheath inlet **450** and supplying focusing fluid SF to the first and second sets of vertical fluid focusing channels **740**, **750**. Additionally, FIGS. **6**A-**6**D illustrate an alternative vertical placement of some portions of each of the first vertical fluid focusing channel **740** and the second vertical fluid focusing channel **750**.

Further, compared to the embodiment of FIGS. **5**A-**5**D, the embodiment of FIGS. **6**A-**6**D are provided with a relatively large cross sectional areas of both the first set of vertical fluid focusing channels **740** and the second set of vertical fluid focusing channels **750**. This greater cross-sectional area provides less resistance to the focusing fluid entering the vertical fluid focusing component **734** relative to the focusing fluid enter the lateral fluid focusing component **732**. Thus, another way to balance and/or control the focusing forces acting on the sample S is provided by controlling the relative fluidic resistances of the focusing fluid flow into the vertical fluid focusing component **734** and into the lateral fluid focusing component **732**.

Even further, the embodiment of FIGS. **6**A-**6**D are provided with an enhanced sheath aggregating volume **422** in other to accommodate the relatively large cross sectional areas of both the first set of vertical fluid focusing channels **740** and the second set of vertical fluid focusing channels **750**. Also of interest is that the vertical fluid focusing channels **740**, **750** are configured with a reduced downstream cross-sectional area (as compared to the greater cross-sectional area provided in the upstream portion of the channels).

Referring back to FIG. **2** and also to FIGS. **3**D, **4**C, **5**C and **6**C, according to certain embodiments, substrate **21** may be formed by bonding or otherwise attaching an upper substrate layer **62** to a lower substrate layer **64**. Referring now to FIG. **7**A, a top view of the substrate **21** is shown with the core stream forming geometry **300** visible through the top layer of the substrate. FIG. **7**B, a lower surface **62***b* of the upper substrate **62** of the substrate of FIG. **7**A is shown. Portions of the fluid focusing components **300'** of the core stream forming geometry **300** are shown provided in the lower surface **62***b*. In FIG. **7**B, a lower surface **62***b* of the upper substrate **62** of the substrate of FIG. **7**A is shown. Complementary portions of the fluid focusing components **300"** core stream forming geometry **300** are shown provided in the lower surface **62***b*. In FIG. **7**C, an upper surface **64***a* of the lower substrate **64** of the substrate of FIG. **7**A is shown. Portions of the fluid focusing components are shown provided in the upper surface **64***a*. The portions of the fluid focusing components **300'**, **300"** provided in these substrate layer surfaces may be provided (via additive or subtractive manufacturing). When the upper surface **64***a* and the lower surface **62***b* are assembled together with the complementary portions of the fluid focusing components aligned with each other, the complete core stream forming geometry is formed. Thus, a complicated core stream forming geometry **300** such as the exemplary core stream forming geometries described herein, may be simply and efficiently provided with just two substrate layers. While the core stream forming geometry **300** depicted in the embodiment in FIGS. **7**A-C is illustrated as the exemplary core stream forming geometry **600** of the embodiment of FIGS. **5**A-D, it is appreciated that an upper substrate layer **62** and a lower substrate layer **64** may similarly be used to define any number of different stream forming geometries including, for example, any of the exemplary stream forming geometries **400**, **500**, **600** and **700** described herein, for example, with respect to the embodiments of FIGS. **3**A-E, **4**A-D, **5**A-D and **6**A-D.

As can be understood from the foregoing, features described for focusing a core stream may be combined with various features for monitoring, detecting, analyzing, and/or sorting particles of interest. See, e.g., U.S. Pat. Nos. 6,877,528, 6,808,075, and 7,298,478, which are hereby incorporated by reference in their entireties.

A system and method for producing a focused sample in a flow channel, such as a micro channel, has been described herein. As can be easily understood from the foregoing, the basic concepts of the present disclosure may be embodied in a variety of ways. As such, the particular embodiments or elements disclosed by the description or shown in the figures accompanying this application are not intended to be limiting, but rather illustrative of the numerous and varied embodiments generically encompassed by the present disclosure or equivalents encompassed with respect to any particular element thereof. In addition, the specific description of a single embodiment or element may not explicitly describe all embodiments or elements possible; many alternatives are implicitly disclosed by the description and figures.

Moreover, for the purposes of the present disclosure, the term "a" or "an" entity refers to one or more of that entity. As such, the terms "a" or "an", "one or more" and "at least one" can be used interchangeably herein.

All numeric values herein are assumed to be modified by the term "about", whether or not explicitly indicated. For the purposes of the present invention, ranges may be expressed as from "about" one particular value to "about" another particular value. It will be understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. When a value is expressed as an approximation by use of the antecedent "about," it will be understood that the particular value forms another embodiment.

We claim:

1. A microfluidic assembly for use with a particle processing instrument, the microfluidic assembly comprising:

a substrate; and

a flow channel formed in the substrate, the flow channel having:

an inlet configured to receive a sample stream;

a fluid focusing region configured to focus the sample stream, the fluid focusing region having a lateral fluid focusing feature, a first vertical fluid focusing feature, and a second vertical fluid focusing feature, the lateral, the first vertical, and the second vertical fluid focusing features provided at different longitudinal locations along the flow channel, wherein a bottom surface of the flow channel lies in a first plane upstream of the first and second vertical fluid focusing features and the bottom surface of the flow channel shifts vertically upward to lie in a second plane downstream of the first and second vertical focusing features; and

an inspection region at least partially downstream of the fluid focusing region.

2. The microfluidic assembly of claim **1**, wherein the lateral fluid focusing feature is configured to introduce focusing fluid into the flow channel symmetrically with respect to a centerline of the sample stream.

3. The microfluidic assembly of claim 2, wherein the first and second vertical fluid focusing features are located downstream of the lateral fluid focusing feature;

wherein the first vertical fluid focusing feature includes a first vertical fluid focusing aperture configured to introduce focusing fluid into the flow channel from above the sample stream; and

wherein the second vertical fluid focusing feature includes a second vertical fluid focusing aperture configured to introduce focusing fluid into the flow channel from below the sample stream.

4. The microfluidic assembly of claim 3, wherein the first vertical fluid focusing aperture is in fluid communication with a first vertical fluid focusing channel and the second vertical fluid focusing aperture is in fluid communication with a second vertical fluid focusing channel.

5. The microfluidic assembly of claim 3, wherein the first vertical fluid focusing aperture is in fluid communication with a first pair of fluid focusing channels;

wherein the second vertical fluid focusing aperture is in fluid communication with a second pair of fluid focusing channels; and

wherein each of the first pair and the second pair of fluid focusing channels are symmetrically arranged with respect to a centerline of the flow channel.

6. The microfluidic assembly of claim 1, wherein the sample stream and the focusing fluid associated with the lateral fluid focusing feature enter the fluid focusing region in a same plane.

7. The microfluidic assembly of claim 1, wherein the fluid focusing region has a varying width upstream of the first and second vertical focusing fluid features; and

wherein the flow channel has a constant width between the first and second vertical focusing fluid features and the inspection region.

8. The microfluidic assembly of claim 1, wherein within the fluid focusing region the fluid flow channel transitions from a first cross section shape to a second cross section shape different from the first cross section shape.

9. The microfluidic assembly of claim 1, wherein each of the fluid focusing features is in fluid communication with a first focusing fluid inlet port provided on a top surface of the substrate.

10. The microfluidic assembly of claim 1, wherein each of the fluid focusing features is in fluid communication with a pair of focusing fluid inlet ports provided on a top surface of the substrate.

11. The microfluidic assembly of claim 1, wherein each of the fluid focusing features introduces a focusing fluid into the flow channel at the different longitudinal locations along the flow channel.

12. A microfluidic chip comprising:

a substantially planar chip substrate having an upper surface and a lower surface;

a microfluidic flow channel provided within the chip substrate;

a first inlet port formed on the upper surface of the chip substrate for receiving a focusing fluid;

wherein the first inlet port is in fluid communication with the microfluidic flow channel,

wherein the microfluidic flow channel includes a first focusing fluid inlet configured to introduce focusing fluid from the first inlet port into the microfluidic channel in a first direction, a second focusing fluid inlet configured to introduce focusing fluid from the first inlet port into the microfluidic channel in a second direction, and a third focusing fluid inlet configured to introduce focusing fluid from the first inlet port into the microfluidic channel in a third direction,

wherein the microfluidic flow channel includes a fluid flow focusing region having an upstream end region and a downstream end region,

wherein the first focusing fluid inlet is configured to introduce focusing fluid into the fluid flow focusing region in the upstream end region,

wherein the second and third focusing fluid inlets are configured to introduce focusing fluid into the fluid flow focusing region in the downstream end region, and

wherein a bottom surface of the microfluidic flow channel lies in a first plane upstream of the second and third focusing fluid inlets and the bottom surface of the microfluidic flow channel lies in a second plane downstream of the second and third focusing fluid inlets.

13. The microfluidic chip of claim 12, further comprising:

a second inlet port formed on the upper surface of the chip substrate for receiving a focusing fluid;

wherein the second inlet port is in fluid communication with the microfluidic flow channel, and

wherein the microfluidic flow channel includes a fourth focusing fluid inlet configured to introduce focusing fluid from the second inlet port into the microfluidic channel in a fourth direction,

wherein the second focusing fluid inlet is configured to introduce focusing fluid from the second inlet port into the microfluidic channel in the second direction, and

wherein the third focusing fluid inlet is configured to introduce focusing fluid from the second inlet port into the microfluidic channel in the third direction.

14. The microfluidic chip of claim 13, wherein the first and fourth focusing fluid inlets are opposed to each other.

15. The microfluidic chip of claim 12, wherein the microfluidic channel lies in a first plane upstream of the second focusing fluid inlet and lies in a second plane downstream of the third focusing fluid inlet.

16. The microfluidic chip of claim 15, wherein the microfluidic channel is formed when a lower surface of an upper substrate layer and an upper surface of a lower substrate layer are directly joined together.

17. A microfluidic chip comprising:

a substantially planar substrate having an upper surface and a lower surface;

a microfluidic channel formed in the substantially planar substrate and having an upper surface and a lower surface;

an inlet port formed on the upper surface of the substantially planar substrate and configured to receive a focusing fluid;

a first focusing fluid channel in fluid communication with the inlet port and configured to introduce focusing fluid into the microfluidic channel via a first aperture in the upper surface of the microfluidic channel; and

a second focusing fluid channel in fluid communication with the inlet port and configured to introduce focusing fluid into the microfluidic channel via a second aperture in the lower surface of the microfluidic channel,

wherein a bottom surface of the microfluidic channel lies in a first plane upstream of the first aperture and the bottom surface of the microfluidic channel lies in a second plane downstream of the second aperture.

18. The microfluidic chip of claim 17, wherein the microfluidic channel and the first and second focusing fluid channels are formed when a lower surface of an upper substrate layer and an upper surface of a lower substrate layer are directly joined together.

19. The microfluidic chip of claim 17, further comprising:

at least one outlet port formed on the upper surface of the substantially planar substrate and in fluid communication with the fluid flow focusing region.

20. The microfluidic chip of claim 17, wherein the first and second focusing fluid channels are located to a first side of a centerline of the microfluidic channel.

21. The microfluidic chip of claim 17, further comprising a third focusing fluid channel in fluid communication with the inlet port and configured to introduce focusing fluid into the microfluidic channel.

22. A microfluidic assembly for use with a particle processing instrument, the microfluidic assembly comprising:

a substrate; and

a flow channel formed in the substrate, the flow channel having:

an inlet configured to receive a sample stream; and

a fluid focusing region configured to focus the sample stream, the fluid focusing region having a lateral fluid focusing feature, a first vertical fluid focusing feature, and a second vertical fluid focusing feature, the lateral, the first vertical, and the second vertical fluid focusing features provided at different longitudinal locations along the flow channel, wherein a top surface of the flow channel lies in a first plane upstream of the first and second vertical fluid focusing features and the top surface of the flow channel shifts vertically upward to lie in a second plane downstream of the first and second vertical focusing features.

23. The microfluidic assembly of claim 22, wherein the lateral fluid focusing feature is configured to introduce focusing fluid into the flow channel symmetrically with respect to a centerline of the sample stream.

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CERTIFICATE OF COMPLIANCE

This brief complies with the type-volume limitations of Federal Rule of Appellate Procedure 32(a)(7)(B) and Federal Circuit Rule 32(b)(1). The brief contains 12,407 words, excluding the parts of the brief exempted by Federal Rule of Appellate Procedure 32(f) and Federal Circuit Rule 32(b)(2).

This brief complies with the typeface requirements of Federal Rule of Appellate Procedure 32(a)(5) and the type style requirements of Federal Rule of Appellate Procedure 32(a)(6). The brief has been prepared in a proportionally spaced typeface using Microsoft Word 365 in 14-point Century Schoolbook font.

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